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=> s antibod?
L1 2528976 ANTIBOD?

=> s l1 and NK cell
L2 18290 L1 AND NK CELL

=> s l2 and stimulate
L3 784 L2 AND STIMULATE

=> s l3 and cytotoxicity
L4 273 L3 AND CYTOTOXICITY

=> s l4 and "hybridoma I-2576"
L5 0 L4 AND "HYBRIDOMA I-2576"

=> s l4 and "AZ20"
L6 0 L4 AND "AZ20"

=> s l4 and "A76"
L7 0 L4 AND "A76"

=> s l4 and "Z25"
L8 0 L4 AND "Z25"

=> s l3 and hybridoma
L9 12 L3 AND HYBRIDOMA

=> dup remove l9
PROCESSING COMPLETED FOR L9
L10 4 DUP REMOVE L9 (8 DUPLICATES REMOVED)

=> d l10 1-4 cbib abs

L10 ANSWER 1 OF 4 MEDLINE on STN
2003317891. PubMed ID: 12847220. Tumor cells engineered with IL-12 and

IL-15 genes induce protective **antibody** responses in nude mice.
Orengo Anna Maria; Di Carlo Emma; Comes Alberto; Fabbi Marina; Piazza Tiziana; Cilli Michele; Musiani Piero; Ferrini Silvano. (Laboratory of Immunopharmacology, Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy.) Journal of immunology (Baltimore, Md. : 1950), (2003 Jul 15) 171 (2) 569-75. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB IL-12 and IL-15 **stimulate** T, B, and **NK cell** functions through independent mechanisms, and cooperative effects of these cytokines have been reported. The human MHC class I-negative small cell lung cancer cell line, N592, genetically engineered to secrete IL-15, N592/IL-15, showed a reduced tumor growth rate, while N592 cells engineered with IL-12, N592/IL-12, grew similarly to the wild-type N592, N592 parental cells (N592pc), in nude mice. However, N592 cells coexpressing both cytokines, N592/IL-12/IL-15 cells, were completely rejected by 100% of nude mice. Here we show that 60% of nude mice rejecting N592/IL-12/IL-15 cells were resistant to N592pc rechallenge. SCID mice rejected N592/IL-12/IL-15 cells, but did not develop resistance to N592pc rechallenge, suggesting a role of Ab responses. Among nude mice rejecting N592/IL-12/IL-15 cells, those developing resistance to N592pc rechallenge had significantly higher titers of anti-N592 IgG2b Abs than nonresistant nude mice. Induction of an Ig class switch in nude mice was related to the expression of IFN-gamma and CD40 ligand in the draining lymph nodes. An IgG2b, anti-N592 mAb, derived from N592/IL-12/IL-15-immunized nude mice splenocytes induced significant protection against N592pc, while an IgM mAb was ineffective. The protective IgG2b mAb, but not the IgM mAb, triggered Ab-dependent cell-mediated cytotoxicity by nude mouse splenocytes against N592pc. These data indicate that IL-12 and IL-15 synergistically trigger innate, immunity-mediated, anti-tumor effects, resulting in cytotoxic IgG Ab responses in T cell-deficient mice. Protective Ab responses may relate to both direct actions of IL-12 and IL-15 on B cells and to the activation of an innate immunity-B cell cross-talk.

L10 ANSWER 2 OF 4 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2003:434340 Document No.: PREV200300434340. Anti-CD30-IL-12 **antibody** -cytokine fusion protein that induces IFN-GAMMA secretion of T cells and **NK cell**-mediated lysis of Hodgkin's lymphoma-derived tumor cells. Heuser, Claudia; Diehl, Volker; Abken, Hinrich; Hombach, Andreas [Reprint Author]. Lab. Tumor Genetics, First Dept. of Internal Medicine, University of Cologne, Josef-Stelzmann-Str. 9, D-50931, Cologne, Germany. andreas.hombach@medizin.uni-koeln.de. International Journal of Cancer, (10 September 2003) Vol. 106, No. 4, pp. 545-552. print. CODEN: IJCNAW. ISSN: 0020-7136. Language: English.

AB Interleukin-12 (IL-12) is a disulfide-linked p40-p35 heterodimeric cytokine and plays a key role in linking innate cellular immunity to an adaptive Th1 response against pathogens and tumor cells and in counteracting a Th2 immune response. The pathogenesis of Hodgkin's disease (HD) is partially attributed to a Th2 dominance associated with functional anergy of T cells that accumulate in the near vicinity to the malignant Hodgkin/Reed-Sternberg (H/RS) cells. To revert Th2 polarization in the tumor lesion, we generated an anti-CD30-IL-12 **antibody** -cytokine fusion protein that binds to CD30 on H/RS cells and is composed of a CD30 binding domain (HRS3-scFv) linked to p40-p35 murine single chain IL-12. The HRS3-scFv-hi-IL-12 fusion protein is expressed as a 110 kD polypeptide, can be purified by affinity chromatography, and has binding specificities to both the CD30 antigen and the IL-12 receptor. After binding to CD30+ H/RS cells, the fusion protein **stimulates** T cells to secrete IFN-gamma, a predominant Th1 cytokine, and induces **NK cells** to lyse CD30+ cells with high efficiency. These properties make the HRS3-scFv-hi-IL-12 fusion protein suitable for the specific immunotherapy of Hodgkin's lymphoma.

L10 ANSWER 3 OF 4 MEDLINE on STN DUPLICATE 1
92287195. PubMed ID: 1599525. Production of natural killer cell activity-augmenting factor (interleukin-6) by human epiphyseal chondrocytes. Malejczyk J; Malejczyk M; Urbanski A; Luger T A. (Department of Histology and Embryology, Warsaw Medical School, Poland.) Arthritis and rheumatism, (1992 Jun) 35 (6) 706-13. Journal code: 0370605. ISSN: 0004-3591. Pub. country: United States. Language: English.

AB OBJECTIVE. We sought to determine the capacity of human epiphyseal chondrocytes to modulate the cytotoxic activity of human natural killer (**NK**) **cells** by determining whether they release interleukin-6 (IL-6), a cytokine recently shown to **stimulate** **NK cell** activity. METHODS. Conditioned medium from human epiphyseal chondrocyte cultures (Ch-CM) was tested for IL-6 activity using the B9 cell **hybridoma** assay. Its **NK** **cell**-stimulating capacity in the presence of K562 (myelogenous leukemia) cells or human chondrocytes was evaluated in a 4-hour 51Cr-release assay. Ch-CM-derived IL-6/**NK cell** -augmenting factor activity was partially purified by high-performance liquid chromatography (HPLC) gel filtration and Western blot. RESULTS. Ch-CM contained an **NK cell**-augmenting factor (NKAF) which was blocked by IL-2 or IL-6 **antibodies**. Ch-CM did not contain detectable IL-2 activity, but it stimulated IL-2 production by human peripheral blood lymphocytes (PBL). This IL-2-inducing capacity was inhibited by IL-6 **antibodies**, indicating that chondrocytes release an IL-6-like activity. Ch-CM significantly enhanced the proliferation of IL-6-dependent B9 **hybridoma** cells, and Western blot analysis of Ch-CM revealed specific bands corresponding to those of highly purified IL-6. Upon HPLC gel filtration, chondrocyte NKAF copurified with chondrocyte IL-6. Pure IL-6 and chondrocyte IL-6 were tested for their ability to **stimulate** the cytotoxic activity of human PBL against chondrocytes. Both mediators significantly enhanced chondrocyte killing. Lysis of chondrocytes by PBL was mediated by **NK cells**, since depletion of CD16+ cells resulted in inhibition of the activity. CONCLUSION. Thus, upon stimulation, chondrocytes produce IL-6 which, through IL-2 induction, augments the activity of **NK cells** against K562 target cells as well as against chondrocytes.

L10 ANSWER 4 OF 4 MEDLINE on STN DUPLICATE 2
92013160. PubMed ID: 1919012. NKR-P1, an activating molecule on rat natural killer cells, **stimulates** phosphoinositide turnover and a rise in intracellular calcium. Ryan J C; Niemi E C; Goldfien R D; Hiserodt J C; Seaman W E. (Department of Medicine, San Francisco Veterans Administration Medical Center, CA 94121.) Journal of immunology (Baltimore, Md. : 1950), (1991 Nov 1) 147 (9) 3244-50. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB NKR-P1 is a 60-kDa homodimer expressed on all rat **NK** **cells**. Previous studies by others suggest that NKR-P1 may play a role in **NK cell** activation because **antibody** to NKR-P1 **stimulates** the release of granules from **NK** **cells**, and anti-NKR-P1 causes redirected lysis by activated **NK cells** against targets that express FcR. To examine the mechanism of transmembrane signaling by NKR-P1, we studied the rat **NK cell** line, RNK-16. We here demonstrate that F(ab')2 **antibody** to NKR-P1 **stimulates** phosphoinositide turnover and a rise in intracellular calcium within RNK-16 cells. The response is augmented by cross-linking the F(ab')2 **antibody**. The phosphoinositide/calcium pathway is also stimulated by NKR-P1 in activated rat **NK cells**, although no response is detectable in polymorphonuclear cells, which also express NKR-P1. We also demonstrate that RNK-16 cells kill the anti-NKR-P1 (3.2.3) **hybridoma** and

that exposure to the **hybridoma** target cells **stimulates** phosphoinositide turnover in RNK-16 cells. Both killing and phosphoinositide turnover are inhibited by F(ab')2 anti-NKR-P1, implicating NKR-P1 in both responses. In contrast, neither cytotoxicity nor phosphoinositide turnover is appreciably blocked by F(ab')2 anti-NKR-P1 in response to YAC-1 targets. Thus, with either target, killing is linked to phosphoinositide turnover, but killing of YAC-1 involves pathways that differ from those that direct killing of the anti-NKR-P1 **hybridoma**. Our studies support the hypothesis that NKR-P1 may serve as an activating cell-surface receptor on **NK cells**, and they clarify the mechanisms by which it activates **NK cells**.

```
=> s hybridoma
L11       67845 HYBRIDOMA

=> s l11 and "I-2576"
L12           1 L11 AND "I-2576"

=> d l12 cbib abs

L12  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2004 ACS on STN
2001:380770   Document No. 135:4465  cDNA encoding novel triggering receptor
     involved in natural cytotoxicity mediated by human natural killer cells,
     and antibodies that identify the same.  Moretta, Alessandro; Bottino,
     Cristina; Biassoni, Roberto (Innate Pharma S.A.S., Fr.; Universita Di
     Genova).  PCT Int. Appl. WO 2001036630 A2 20010525, 83 pp.  DESIGNATED
     STATES: W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH,
     CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU,
     ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA,
     MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
     SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG,
     KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK,
     ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
     TG, TR.  (English).  CODEN: PIXXD2.  APPLICATION: WO 2000-EP11697
     20001115.  PRIORITY: US 1999-440514 19991115; CA 1999-2288307 19991115.
AB   The invention relates to a cDNA sequence encoding a novel receptor termed
     NKp30 of human.  The receptor selectively expressed by all mature NK cells
     and that is involved in human natural cytotoxicity as an activatory
     receptor, to new antibodies that bind to the NKp30 structure, and to the
     pharmaceutical and medicinal uses thereof.


=> s l2 and "AZ20"
L13           0 L2 AND "AZ20"

=> s l2 and "A76"
L14           0 L2 AND "A76"

=> s L2 and "Z25"
L15           0 L2 AND "Z25"

=> s l2 and "p30"
L16           3 L2 AND "P30"

=> dup remove l16
PROCESSING COMPLETED FOR L16
L17           1 DUP REMOVE L16 (2 DUPLICATES REMOVED)

=> d l17 cbib abs
```

L17 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1
89035487. PubMed ID: 2460541. Induction of antigen-specific parasiticidal cytotoxic T cell splenocytes by a major membrane protein (**P30**) of Toxoplasma gondii. Khan I A; Smith K A; Kasper L H. (Department of Medicine, Dartmouth Medical School, Hanover, NH 03756.) Journal of immunology (Baltimore, Md. : 1950), (1988 Nov 15) 141 (10) 3600-5. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Infection with Toxoplasma gondii has become a major cause of morbidity in patients with AIDS. To investigate the mechanisms responsible for immune responses to toxoplasma Ag we used a highly purified membrane protein (**P30**) of T. gondii to stimulate an in vitro Ag-specific cytotoxic T cell response. **P30** immune mouse splenocytes reduced extracellular T. gondii plaque-forming units by more than 50% when incubated at an E/T ratio of 10:1 or greater. By using a [3H]uracil radioisotope release assay, the effect of the immune splenocytes was determined to be a direct parasite lytic mechanism. The immune splenocytes were **P30** Ag specific and of the Thy 1.2, Lyt2,3+ (CD4-, CD8+) phenotype, specific for mouse cytotoxic T cells. Opsonization of the parasites with monoclonal **P30**-reactive mAb did not enhance parasiticidal activity. Culture supernatants obtained during the 2-h cytotoxic assay were not parasiticidal, and anti-asialo-GM1 **antibody** plus C did not destroy the parasiticidal activity of the **P30** responder cells. Accordingly, we have identified an Ag-specific subset of CD4-, CD8+, **P30** responder T cells that are directly parasiticidal to extracellular T. gondii, and that exhibit cytotoxicity independent of **antibody** opsonization, lymphokine secretion, **NK cell** activity, and, apparently, MHC involvement as well.

```
=> s "anti-p30"
L18         320 "ANTI-P30"

=> s l18 and NK cell
L19           0 L18 AND NK CELL

=> s l18 and NK receptor
L20           0 L18 AND NK RECEPTOR

=> s method
L21    12290543 METHOD

=> s l21 and antibod?
L22      500695 L21 AND ANTIBOD?

=> s l22 and stimulat?
L23       32438 L22 AND STIMULAT?

=> s l23 and NK activity
L24          79 L23 AND NK ACTIVITY

=> s l24 and NK receptor
L25           0 L24 AND NK RECEPTOR

=> dup remove l24
PROCESSING COMPLETED FOR L24
L26          38 DUP REMOVE L24 (41 DUPLICATES REMOVED)

=> d l26 1-38 cbib abs

L26  ANSWER 1 OF 38     MEDLINE on STN                  DUPLICATE 1
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2004049609. PubMed ID: 14750556. PCBs, hexachlorobenzene and DDE are not associated with recurrent miscarriage. Sugiura-Ogasawara Mayumi; Ozaki Yasuhiko; Sonta Shin-ichi; Makino Tsunehisa; Suzumori Kaoru. (Department of Obstetrics and Gynecology, Nagoya City University Medical School, Nagoya, Japan.. og.mym@med.nagoya-cu.ac.jp) . American journal of reproductive immunology (New York, N.Y. : 1989), (2003 Dec) 50 (6) 485-9. Journal code: 8912860. ISSN: 1046-7408. Pub. country: Denmark. Language: English.

AB PROBLEM: A case-control study was designed to evaluate any associations between high exposure to polychlorinated biphenyls (PCB), hexachlorobenzene (HCB) and the 1,1,1,-trichloro-2,2-bis (p-chlorophenyl) ethane (DDT) metabolite 1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene (DDE) and recurrent miscarriage and immunoendocrine abnormalities. **METHODS** OF STUDY: A total of 18 kinds of co-planer PCBs, HCB, DDE, natural killer cell (**NK**) **activity**, antiphospholipid **antibodies**, antinuclear **antibody**, prolactin, progesterone, thyroid-**stimulating** hormone (TSH) and free T4 were examined in 45 patients with a history of three or more (3-11) consecutive first-trimester miscarriages and 30 healthy women with no history of live birth and infertility. RESULTS: There were no differences in mean +/- S.D. values in serum samples for PCBs, HCB and DDE between patients and controls. Hypothyroidism, hyperprolactinemia, luteal phase defects, NK cell activity and the presence of autoantibodies were also not associated with levels of any of the compounds in the patients. CONCLUSION: PCBs, HCB and DDE are not associated with miscarriage and immunoendocrine abnormalities in patients with a history of recurrent miscarriage.

L26 ANSWER 2 OF 38 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

2003:1045658 The Genuine Article (R) Number: 747JP. PCBs, hexachlorobenzene and DDE are not associated with recurrent miscarriage. Sugiura-Ogasawara M (Reprint); Ozaki Y; Sonta S; Makino T; Suzumori K. Nagoya City Univ, Sch Med, Dept Obstet & Gynecol, Mizuho Ku, Nagoya, Aichi 4678601, Japan (Reprint); Aichi Human Serv Ctr, Dept Genet, Inst Dev Res, Kasugai, Aichi, Japan; Tokai Univ, Sch Med, Dept Obstet & Gynecol, Kanagawa 2591100, Japan . AMERICAN JOURNAL OF REPRODUCTIVE IMMUNOLOGY (DEC 2003) Vol. 50, No. 6, pp. 485-489. Publisher: BLACKWELL MUNKSGAARD. 35 NORRE SOGADE, PO BOX 2148, DK-1016 COPENHAGEN, DENMARK. ISSN: 8755-8920. Pub. country: Japan. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB PROBLEM: A case-control study was designed to evaluate any associations between high exposure to polychlorinated biphenyls (PCB), hexachlorobenzene (HCB) and the 1,1,1,-trichloro-2,2-bis (p-chlorophenyl) ethane (DDT) metabolite 1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene (DDE) and recurrent miscarriage and immunoendocrine abnormalities.

METHODS OF STUDY: A total of 18 kinds of co-planer PCBs, HCB, DDE, natural killer cell (**NK**) **activity**, antiphospholipid **antibodies**, antinuclear **antibody**, prolactin, progesterone, thyroid-**stimulating** hormone (TSH) and free T4 were examined in 45 patients with a history of three or more (3-11) consecutive first-trimester miscarriages and 30 healthy women with no history of live birth and infertility.

RESULTS: There were no differences in mean +/- S.D. values in serum samples for PCBs, HCB and DDE between patients and controls. Hypothyroidism, hyperprolactinemia, luteal phase defects, NK cell activity and the presence of autoantibodies were also not associated with levels of any of the compounds in the patients.

CONCLUSION: PCBs, HCB and DDE are not associated with miscarriage and immunoendocrine abnormalities in patients with a history of recurrent miscarriage..

L26 ANSWER 3 OF 38 MEDLINE on STN DUPLICATE 2

2003212307. PubMed ID: 12733588. Progesterone regulates IL12 expression in

pregnancy lymphocytes by inhibiting phospholipase A2. Par G; Geli J; Kozma N; Varga P; Szekeres-Bartho J. (Department of Medical Microbiology and Immunology, Medical School, Pecs University, Pecs, Hungary.) American journal of reproductive immunology (New York, N.Y. : 1989), (2003 Jan) 49 (1) 1-5. Journal code: 8912860. ISSN: 1046-7408. Pub. country: Denmark. Language: English.

AB PROBLEM: Progesterone-induced blocking factor (PIBF) is one of the pathways that mediate the immunological effects of progesterone. PIBF inhibits natural killer (NK) cytotoxic activity. Recently we showed that neutralization of PIBF results in an increased interleukin (IL)-12 expression, which is corrected by cyclooxygenase inhibitors. As exogenous arachidonic acid (AA) voids the NK blocking effect of PIBF, it is likely that PIBF acts before the level of the cyclooxygenase enzyme. Therefore in this study we investigated the effect of PIBF neutralizing **antibody** and simultaneous phospholipase A2 inhibitor quinacrine (Q) treatment on IL-12 production. **METHODS**: Pregnancy lymphocytes were treated with anti-PIBF **antibody** or lipopolysaccharide (LPS) as a positive control, in the presence or absence of Q. IL-12 expression by PBMC was detected by immunocytochemistry. RESULTS: Neutralization of PIBF as well as LPS treatment resulted in an increased IL-12 expression, which was corrected by simultaneous Q treatment. Pre-treatment of lymphocytes with progesterone prevented the **stimulating** effect of LPS on IL-12 production. CONCLUSION: Progesterone binding of the lymphocytes is followed by the release of PIBF that inhibits AA release. The subsequent block of prostaglandin synthesis reduces IL-12 production and results in a lowered cytotoxic **NK activity**, which may contribute to a normal pregnancy outcome.

L26 ANSWER 4 OF 38 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

2003:189933 The Genuine Article (R) Number: 648PP. Progesterone regulates IL12 expression in pregnancy lymphocytes by inhibiting phospholipase A2. Par G; Geli J; Kozma N; Varga R; Szekeres-Bartho J (Reprint). Univ Pecs, Sch Med, Dept Med Microbiol & Immunol, Szigeti Ut 12, H-7643 Pecs, Hungary (Reprint); Univ Pecs, Sch Med, Dept Med Microbiol & Immunol, H-7643 Pecs, Hungary; Cty Hosp, Dept Obstet & Gynecol, Pecs, Hungary. AMERICAN JOURNAL OF REPRODUCTIVE IMMUNOLOGY (JAN 2003) Vol. 49, No. 1, pp. 1-5. Publisher: BLACKWELL MUNKSGAARD. 35 NORRE SOGADE, PO BOX 2148, DK-1016 COPENHAGEN, DENMARK. ISSN: 8755-8920. Pub. country: Hungary. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB PROBLEM: Progesterone-induced blocking factor (PIBF) is one of the pathways that mediate the immunological effects of progesterone. PIBF inhibits natural killer (NK) cytotoxic activity. Recently we showed that neutralization of PIBF results in an increased interleukin (IL)-12 expression, which is corrected by cyclooxygenase inhibitors. As exogenous arachidonic acid (AA) voids the NK blocking effect of PIBF, it is likely that PIBF acts before the level of the cyclooxygenase enzyme. Therefore in this study we investigated the effect of PIBF neutralizing **antibody** and simultaneous phospholipase A2 inhibitor quinacrine (Q) treatment on IL-12 production.

METHODS: Pregnancy lymphocytes were treated with anti-PIBF **antibody** or lipopolysaccharide (LPS) as a positive control, in the presence or absence of Q. IL-12 expression by PBMC was detected by immunocytochemistry.

RESULTS: Neutralization of PIBF as well as LPS treatment resulted in an increased IL-12 expression, which was corrected by simultaneous Q treatment. Pre-treatment of lymphocytes with progesterone prevented the **stimulating** effect of LPS on IL-12 production.

CONCLUSION: Progesterone binding of the lymphocytes is followed by the release of PIBF that inhibits AA release. The subsequent block of prostaglandin synthesis reduces IL-12 production and results in a lowered cytotoxic **NK activity**, which may contribute to a normal pregnancy outcome.

L26 ANSWER 5 OF 38 MEDLINE on STN DUPLICATE 3
2003010274. PubMed ID: 12516630. Progesterone induced blocking factor (PIBF) mediates progesterone induced suppression of decidual lymphocyte cytotoxicity. Laskarin Gordana; Tokmadzic Vlatka S; Strbo Natasa; Bogovic Tatjana; Szekeres-Bartho Julia; Randic Ljiljana; Podack Eckhard R; Rukavina Daniel. (Department of Physiology and Immunology, Medical Faculty, University of Rijeka, Rijeka, Croatia.) American journal of reproductive immunology (New York, N.Y. : 1989), (2002 Oct) 48 (4) 201-9. Journal code: 8912860. ISSN: 1046-7408. Pub. country: Denmark. Language: English.

AB PROBLEM: Progesterone induced blocking factor (PIBF) is a mediator of progesterone that blocks peripheral blood lytic natural killer (**NK**) **activity**. Progesterone or PIBF **stimulated** decidual macrophages block up-regulation of perforin expression in decidual lymphocytes (DL). Therefore, we investigated whether progesterone regulates cytotoxicity of DL. **METHOD** OD STUDY: Decidual mononuclear cells were cultured with progesterone. PIBF, progesterone and anti-PIBF **antibody** or in the medium only. Cytolytic activity of non-adherent DL was measured by PKH-26 (red) 2 hr cytolytic assay and flow cytometry. Perforin positive DL were detected by immunofluorescency and PIBF-positive cells by immunohistology. RESULTS: Progesterone and PIBF, in a dose-dependent manner decreased cytotoxicity of DL against K-562 targets, and perforin egzocytosys was blocked. Anti-PIBF **antibodies** reversed the progesterone mediated reduction in cytolytic activity of DL. PIBF positive cells were found in first trimester pregnancy decidua. CONCLUSION: The results indicate possible role for PIBF, as a mediator of progesterone in regulation of DL cytolytic activity at the maternal-foetal (M-F) interface.

L26 ANSWER 6 OF 38 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2002:783785 The Genuine Article (R) Number: 596VP. Progesterone induced blocking factor (PIBF) mediates progesterone induced suppression of decidual lymphocyte cytotoxicity. Laskarin G; Tokmadzic V S; Strbo N; Bogovic T; Szekeres-Bartho J; Randic L; Podack E R; Rukavina D (Reprint). Univ Rijeka, Dept Physiol & Immunol, Fac Med, B Branchetta 20-a, HR-51000 Rijeka, Croatia (Reprint); Univ Rijeka, Dept Physiol & Immunol, Fac Med, HR-51000 Rijeka, Croatia; Sch Med, Dept Microbiol & Immunol, Pecs, Hungary; Univ Rijeka, Dept Obstet & Gynaecol, Fac Med, HR-51000 Rijeka, Croatia; Miami Univ, Dept Microbiol & Immunol, Sch Med, Miami, FL USA. AMERICAN JOURNAL OF REPRODUCTIVE IMMUNOLOGY (OCT 2002) Vol. 48, No. 4, pp. 201-209. Publisher: BLACKWELL MUNKSGAARD. 35 NORRE SOGADE, PO BOX 2148, DK-1016 COPENHAGEN, DENMARK. ISSN: 8755-8920. Pub. country: Croatia; Hungary; USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB PROBLEM: Progesterone induced blocking factor (PIBF) is a mediator of progesterone that blocks peripheral blood lytic natural killer (**NK**) **activity**. Progesterone or PIBF **stimulated** decidual macrophages block up-regulation of perforin expression in decidual lymphocytes (DL). Therefore, we investigated whether progesterone regulates cytotoxicity of DL.

METHOD OD STUDY: Decidual mononuclear cells were cultured with progesterone, PIBF, progesterone and anti-PIBF **antibody** or in the medium only. Cytolytic activity of non-adherent DL was measured by PKH-26 (red) 2 hr cytolytic assay and flow cytometry. Perforin positive DL were detected by immunofluorescency and PIBF-positive cells by immunohistology.

RESULTS: Progesterone and PIBF, in a dose-dependent manner decreased cytotoxicity of DL against K-562 targets, and perforin egzocytosys was blocked. Anti-PIBF **antibodies** reversed the progesterone mediated reduction in cytolytic activity of DL. PIBF positive cells were found in first trimester pregnancy decidua.

CONCLUSION: The results indicate possible role for PIBF, as a mediator of progesterone in regulation of DL cytolytic activity at the maternal-foetal (M-F) interface.

L26 ANSWER 7 OF 38 MEDLINE on STN
2002150128. PubMed ID: 11882356. Upregulation of natural killer cells functions underlies the efficacy of intratumorally injected dendritic cells engineered to produce interleukin-12. Rodriguez-Calvillo Mercedes; Duarte Marina; Tirapu Inigo; Berraondo Pedro; Mazzolini Guillermo; Qian Chen; Prieto Jesus; Melero Ignacio. (Gene Therapy Unit, Department of Internal Medicine, University of Navarra, Pamplona, Spain.) Experimental hematology, (2002 Mar) 30 (3) 195-204. Journal code: 0402313. ISSN: 0301-472X. Pub. country: Netherlands. Language: English.

AB OBJECTIVE: Injection of dendritic cells (DC) engineered with recombinant adenoviral vectors to produce interleukin-12 (IL-12) inside experimental murine tumors frequently achieves complete regressions. In such a system the function of CD8(+) T cells has been shown to be an absolute requirement, in contrast to observations made upon depletion of CD4(+) T cells, which minimally affected the outcome. The aim of this work was to study the possible involvement of natural killer (NK) cells in this setting. MATERIALS, **METHODS**, AND RESULTS: Depletions with anti-AsialoGM1 antiserum showed only a small decrease in the proportion of complete regressions obtained that correlated with induction of **NK activities** in lymphatic tissues into which DC migrate, whereas combined depletions of CD4(+) and NK cells completely eliminated the antitumor effects. Likewise in vivo neutralization of interferon-gamma (IFN-gamma) also eliminated those therapeutic effects. Trying to define the cellular role played by NK cells in vivo, it was observed that injection of cultured DC inside the spleen of T- and B-cell-deficient (Rag1(-/-)) mice induced upregulation of **NK activity** only if DC had been adenovirally engineered to produce IL-12. In addition, identically transfected fibroblasts also activated NK cells, indicating that IL-12 transfection was the unique requirement. Equivalent human DC only activated in vitro the cytolytic and cytokine-secreting functions of autologous NK cells if transfected to express human IL-12. CONCLUSIONS: Overall, these results point out an important role played by NK cell activation in the potent immunotherapeutic effects elicited by intratumoral injection of IL-12--secreting DC and that NK activation under these conditions is mainly, if not only, dependent on IL-12.

L26 ANSWER 8 OF 38 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2001:275738 Document No.: PREV200100275738. Augmentation of antitumor effects by NK cell inhibitory receptor blockade in vitro and in vivo. Koh, Crystal Y.; Blazar, Bruce R.; George, Thaddeus; Welniak, Lisbeth A.; Capitini, Christian M.; Raziuddin, Arati; Murphy, William J. [Reprint author]; Bennett, Michael. SAIC-Frederick, Bldg 567, Rm 210, Frederick, MD, 21702, USA. murphyw@ncifcrf.gov. Blood, (May 15, 2001) Vol. 97, No. 10, pp. 3132-3137. print.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Subsets of natural killer (NK) cells are characterized by the expression of inhibitory and/or **stimulatory** receptors specific for major histocompatibility complex (MHC) class I determinants. In mice, these include the Ly49 family of molecules. One mechanism by which tumor cells may evade NK cell killing is by expressing the appropriate MHC class I and binding inhibitory Ly49 receptors. Therefore, the question of whether blocking the interaction between the Ly49 inhibitory receptors on NK and MHC class I cells on tumor cells augments antitumor activity was investigated. Blockade of Ly49C and I inhibitory receptors using F(ab')2 fragments of the 5E6 monoclonal **antibody** (mAb) resulted in increased cytotoxicity against syngeneic tumors and decreased tumor cell growth in vitro. The effect of 5E6 F(ab')2 was specific for the MHC of the tumor, as the use of F(ab')2 of the mAb against Ly49G2 failed to

increase **NK activity**. Treatment of leukemia-bearing mice With 5E6 F(ab')2 fragments or adoptive transfer of NK cells treated ex vivo with the F(ab')2 resulted in significant increases in survival. These results demonstrate that blockade of NK inhibitory receptors enhances antitumor activity both in vitro and in vivo, suggesting that NK inhibitory receptors can be responsible for diminishing antitumor responses. Therefore, strategies to block inhibitory receptors may be of potential use in increasing the efficacy of immunotherapy.

L26 ANSWER 9 OF 38 MEDLINE on STN DUPLICATE 4
2001699713. PubMed ID: 11747352. Peripheral blood dendritic cells, but not monocyte-derived dendritic cells, can augment human NK cell function. Osada T; Nagawa H; Kitayama J; Tsuno N H; Ishihara S; Takamizawa M; Shibata Y. (Department of Transfusion Medicine, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-8655, Japan.) Cellular immunology, (2001 Oct 10) 213 (1) 14-23. Journal code: 1246405. ISSN: 0008-8749. Pub. country: United States. Language: English.

AB Dendritic cells (DCs) are essential antigen-presenting cells with a wide variety of functions relating to both adaptive and innate immunity. Recently, interactions of DCs with natural killer (NK) cells and NK1.1-positive T cells have been reported in mice. However, in humans, this interaction is not well understood. Here we report the use of a coculture **method** to analyze the modulation of NK cell function in antitumor immunity by DCs. We found that peripheral blood DCs (PDCs) enhanced NK cell activity in cytotoxicity assay, even without direct contact between DC and NK cells. In contrast, neither monocyte-derived DCs (MoDCs), nor TNF-alpha-treated MoDCs, **stimulated** NK lytic activity. Secretion of IL-12 and TNF-alpha into the PDC-NK coculture supernatant was increased. However, blocking **antibodies** against these cytokines could not completely abolish the upregulation of **NK activity**, suggesting the presence of other soluble factor(s) that affect DC-NK cell interaction. To summarize, this study demonstrates for the first time the direct activation of human NK cells by DC-NK cell interaction in vitro, suggesting that DCs may have a central role linking the innate and adaptive immune responses. Moreover, in **stimulating** NK cell function, PDCs appear to have a different potential from MoDCs.

L26 ANSWER 10 OF 38 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2001:304957 Document No.: PREV200100304957. Antileukemic activity and risk of relapse after allogeneic transplantation of purified peripheral CD34+ stem cells in children. Lang, P. [Reprint author]; Klingebiel, T. [Reprint author]; Pfeiffer, M. [Reprint author]; Eyrich, M. [Reprint author]; Stanjevic, S. [Reprint author]; Schumm, M. [Reprint author]; Schlegel, P. G. [Reprint author]; Greil, J. [Reprint author]; Bader, P. [Reprint author]; Handgretinger, R.. University Children's Hospital, Tuebingen, Germany. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 582a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology. San Francisco, California, USA. December 01-05, 2000. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB 52 pediatric patients suffering from high risk malignancies (acute lymphatic leukemias (26), acute myeloic leukemias (9), MDS (4), CML (7), JMML (2) and lymphomas (4); 21 patients were not in remission), were transplanted with highly purified CD34+ stem cells from matched unrelated donors (n=21) or from 1-3 HLA loci mismatched parental donors (n=31) in order to prevent acute and chronic GvHD and to avoid any posttransplant pharmacological immunosuppression. Isolation of stem cells was performed with the MACS **method** with a median purity of 98.7% CD34+ and with a profound depletion of T-cells (median 9X103/kg body weight). After

transplantation, T-cell recovery was delayed (median time to reach >100 T-cells/mul was 96 days) and T-cell response to mitogen **stimulation** was reduced. T-cell add backs were not given regularly. However, high numbers of Natural Killer (NK)-cells were detectable in all patients already in the first month after transplantation. Cytotoxic activity of NK-cells against targets with low HLA class I expression (K562, fresh leukemic blasts) was normal or increased in most patients, and could be further enhanced by Interleukin (IL)2. Additionally, NK-cells were able to lyse fresh leukemic blasts with high HLA class I expression in the ADCC (**antibody** dependent cellular cytotoxicity). Clinical observations: 18/52 patients relapsed (median follow up 2 years, range 7 months-4.2 years). The actuarial risk of relapse was compared with that of a historical group of patients (n=28) from our institution, receiving unmanipulated (non T-cell depleted) bone marrow from unrelated donors and pharmacological GvHD prophylaxis. No significant difference was found (CD34+ enriched group: 45.3% risk of relapse after 2 years, unmanipulated group: 45.7%). The 2 year-overall survival was 35.6% in the CD34+ enriched group and 32.1% in the unmanipulated group. Furthermore, **NK activity** was monitored in 25 patients after transplantation with purified CD34 + stem cells with a longest follow up of 3 years up to now: patients with constantly low **NK activity** showed significantly more lethal infections and relapses than patients with normal or increased cytotoxic levels (alpha=0.05). In conclusion, allogeneic transplantation of highly purified CD34+ stem cells shows no increased risk of relapse in our pediatric patients at this time despite of profound T-cell depletion. This might be due to the high number of transplanted CD34+ stem cells and to the absence of any posttransplant pharmacological immunosuppression which lead to a rapid NK-cell recovery and good **NK activity**. Constantly low **NK activity** levels in some patients were associated with significantly more lethal infections and relapses.

L26 ANSWER 11 OF 38 MEDLINE on STN

1999459115. PubMed ID: 10527955. Immunologic and virologic studies in long-term nonprogressors with HIV-1 infection. Mendila M; Heiken H; Becker S; Stoll M; Kemper A; Jacobs R; Schmidt R E. (Department of Medicine, Division of Clinical Immunology, Hannover Medical School, D-30625 Hannover, Germany. immunologie@mh-hannover. de.) European journal of medical research, (1999 Oct 15) 4 (10) 417-24. Journal code: 9517857. ISSN: 0949-2321. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB BACKGROUND: It is not known whether clinical latency in long-term nonprogressors (LTNP) with HIV-1 infection is due to a strong HIV-1 specific immune response of the host or to virologic factors. - **METHODS**: Peripheral blood mononuclear cells (PBMC) of 6 LTNP were analyzed for their phenotype, proliferation rates, natural killer (NK) cell activity, **antibody** dependent cellular cytotoxicity (ADCC), and CCR5 chemokine receptor genotype. Furthermore sequence analyses of the HIV-1 gene were performed. - RESULTS: Phenotypic analyses of lymphocyte subsets revealed increased CD8+ as well as HLA-DR+ expressing cells in LTNP and patients with progressive disease (PRO). Proliferation assays in LTNP and PRO showed a reduction of **stimulation** by polyclonal mitogens (PHA, ConA, PWM) of up to 60%. **NK activity** was within normal ranges in LTNP but reduced in PRO. 1 LTNP exhibited heterozygosity for CCR5-D32. A mutant HIV nef gene was not discovered by PCR in any of the LTNP. HIV-V3 loop PCR in 5 LTNP revealed the HIV-1B (NSI) subtype. In 2 patients further sequence analyses of the HIV-1 genome showed homozygous mutations in the Sp1 and NF-kB binding sites. CONCLUSION: The non-progression of HIV-1 infection in some LTNP seems to be due to single mutations in the viral genome resulting in a less replicative HIV-1 subtype or to a mutant chemokine receptor leading

to a reduced HIV-1 entry into CD4+ cells. NK cell activity might be an additional contributing factor in controlling viremia.

L26 ANSWER 12 OF 38 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

1999254135 EMBASE Biological features of human T-activated killer cells. Wei H.; Su H.; Yao X.. H. Wei, Institute of Hematology, Lanzhou Medical College, Dong-Gang West Road, Lanzhou 730000, Gansu Province, China. Chinese Journal of Cancer Research 11/2 (111-114) 1999.
Refs: 8.
ISSN: 1000-9604. CODEN: CJCRFH. Pub. Country: China. Language: English. Summary Language: English.

AB Objective: To investigate the immunobiological essence of T-activated killer (T-AK) cells induced by anti-CD3 monoclonal **antibody** (CD3McAb) and recombinant interleukin-2 (rIL-2) co-**stimulation**. **Methods**: The cytomorphology, phenotype and cytotoxicity of T-AK cells generated from human peripheral blood mononuclear cells (PBMC) were determined. Results: T-AK cells were similar to activated lymphoblasts in morphology, more than 90% of T-AK cells expressed the phenotypes of T-lymphocytes (CD3+, CD8+), and 20%-50% of the cells were NK-like phenotype (CD16+, CD56+), some of them expressed IL-2 receptor (CD25+), CD38 antigen (CD38+) and MHC- II antigen (HLA-DR+) characteristic marks for the activated T lymphocytes. T-AK cells attacking targets were morphologically large volumes with granules and mainly contained CD8+ and CD56+ cells. TAK cells possessed high tumoricidal activities against NK-sensitive K562 cells and NK-resistant Raji cells, the cytotoxicity was composed of mainly CD3McAb-activated CD3AK activity (.apprx.50%), IL-2 induced LAK activity (.apprx.30%), **NK activity** (.apprx.10%) and the activities of inhibitory factors in T-AK supernatant (.apprx.10%). Conclusion: T-AK cells are a heterogeneous cell population consisting of mainly activated T lymphocytes and NK-like cells, the main part of T-AK cytotoxicity is the common activities of CD3AK cells and LAK cells.

L26 ANSWER 13 OF 38 MEDLINE on STN DUPLICATE 5

1998264322. PubMed ID: 9603183. Xenospecific CD8+ cytotoxic T lymphocyte generation: accessory function for CD4+ T cells and natural killer 1.1+ cells. Smyth M J; Kershaw M H; Darcy P K. (Cellular Cytotoxicity Laboratory, The Austin Research Institute, Heidelberg, Victoria, Australia.) Transplantation, (1998 May 15) 65 (9) 1278-81. Journal code: 0132144. ISSN: 0041-1337. Pub. country: United States. Language: English.

AB BACKGROUND: Controversy exists as to whether natural killer (NK)1.1+ cells additionally support cytotoxic T lymphocyte (CTL) generation. We have previously demonstrated that mice generate a strong in vitro xenospecific CTL response in local popliteal lymph nodes (LN) to footpad immunizations with large numbers of human tumor cells. **METHODS**: In vivo depletion of various LN subsets using cytotoxic monoclonal **antibodies** was used to determine their relative importance in **stimulating** xenospecific CD8+ CTL responses to human Jurkat tumor cells. Depletion of functional NK cells in vivo was evidenced by the relative lack of NK1.1+ cells and **NK activity** in the spleens and LN of anti-NK1.1 monoclonal **antibody**-treated mice. CONCLUSION: Depletion of LN subsets indicated that CD4+ T cells were critical in generating an effective xenospecific CD8+ CTL response, but also suggested that NK1.1+ cells play a significant additional accessory role in the development of mouse anti-human xenospecific CTL.

L26 ANSWER 14 OF 38 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

1998:480458 Document No.: PREV199800480458. Kinetics of in vitro natural killer activity against K562 cells as detected by flow cytometry. Zamai, Loris; Mariani, Adriana R.; Zauli, Giorgio; Rodella, Luigi; Rezzani, Rita; Manzoli, Francesco A.; Vitale, Marco [Reprint author]. Dep. Biomed. Sci.

Biotechnol., Human Anat. Section, via Valsabbina 19, 25123 Brescia, Italy. Cytometry, (Aug. 1, 1998) Vol. 32, No. 4, pp. 280-285. print.
CODEN: CYTODQ. ISSN: 0196-4763. Language: English.

AB Natural killer (NK) cells bind to K562 tumor target cells in vitro and kill them. The binding and cytotoxic activities of NK cells are tightly related to each other: degranulation of the cytotoxic effector is the basis for target cell damage and a consequence of effector-target recognition and binding. However, the two phases of **NK activity**, binding and killing, have always been measured separately by various methodologies and under different experimental conditions, because of the lack of a comprehensive methodology able to measure both of them at one time. Here we describe the simultaneous measurement of the binding and killing activities against K562 of resting and cytokine (IL-2 or IL-12)-**stimulated** NK cells by flow cytometry. NK, K562 and conjugates can be identified and measured by flow cytometry on the basis of NK mAb staining and target cells autofluorescence (Binding Plot). Within each population of the binding plot, killed targets can be identified and measured by their scatter characteristics (Cytotoxicity Plot). We show that i) the conjugate formation is enhanced in cytokine-**stimulated** cells, even at relatively short co-incubation times; ii) the conjugate release is also accelerated by cytokines; iii) the conjugate release is always quicker than the induction of the morphological changes in the target cell that generate its modified scattering properties.

L26 ANSWER 15 OF 38 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1998:214439 Document No.: PREV199800214439. Immunization of mice with melanoma cells transfected to secrete the superantigen, staphylococcal enterotoxin A. Shrayer, David P. [Reprint author]; Kouttab, Nicolas; Hearing, Vincent J.; Wanebo, Harold J. [Reprint author]. Dep. Surg., Roger Williams Med. Cent., Brown Univ., 825 Chalkstone Ave., Providence, RI 02908, USA. Cancer Immunology Immunotherapy, (March, 1998) Vol. 46, No. 1, pp. 7-13. print.
CODEN: CIIMDN. ISSN: 0340-7004. Language: English.

AB Immunization of mice with a melanoma vaccine coupled with staphylococcal enterotoxin A (SEA) inhibits the growth of primary melanoma tumors in mice. We have now successfully transfected B16 cells with the sea gene and have immunized C57BL/6 mice subcutaneously once per week for 4 weeks prior to tumor challenge with vaccines of irradiated B16 cells or, 4 weeks following tumor challenge of naive mice with B16 cells, with irradiated B16 cells transfected with the sea gene. Primary tumor growth following both types of treatments was inhibited significantly. To characterize immune responses to these immunogens, we examined the production of **antibodies** to the B700 melanoma antigen, the **stimulation** of endogenous IL-2 production, the expression of CD4, CD8, Vbeta and CD25 T cell markers, and the induction of **NK activity**. At 4 weeks following immunization of mice, there was a significant increase ($P < 0.05$) in levels of interleukin-2 production by splenocytes from mice immunized with SEA-secreting B16 cells or with the parental B16 cells, compared to controls. Levels of **antibodies** to the B700 melanoma antigen were also significantly higher in mice immunized with the SEA-secreting B16 cells, as was expression of CD4, CD8, CD25 and VP T cell antigens, particularly CD4. Natural killer cell activity (at various E:T ratios) was tenfold higher in splenocytes of mice immunized with SEA-secreting B16 cells, and fivefold higher in mice immunized with the parental B16 cells, compared to controls. These data confirm the possibility of using irradiated murine melanoma cells transfected to secrete SEA in vaccines targeted at preventing the development and growth of melanoma.

L26 ANSWER 16 OF 38 MEDLINE on STN DUPLICATE 6
97205681. PubMed ID: 9138456. Human cytotrophoblastic cells absorb the NK blocking activity of monoclonal BA11. Cadavid A P; Guilbert L J; Jalali G

R; Underwood J L; Mowbray J F; Clark D A. (Reproduction Program, University of Antioquia, Medellin, Colombia, SA.) American journal of reproductive immunology (New York, N.Y. : 1989), (1997 Jan) 37 (1) 73-8. Journal code: 8912860. ISSN: 1046-7408. Pub. country: Denmark. Language: English.

AB PROBLEM: R80K is a polymorphic alloantigeneic protein present on human placental trophoblast and on paternal B lymphocytes and monocytes. This protein, unlike the former candidate TLX antigen, **stimulates** a protective maternal immune response in vivo. A murine monoclonal BA11 **antibody**, directed against R80K, prevents abortion in three murine pregnancy-failure models and inhibits human and murine **NK activity**. We attempted to define the target of BA11 in the human NK assay system. **METHODS**: A CELISA **method** was used to detect R80K antigen on the surface of different cells using the BA11 **antibody**. The effect, on human peripheral blood **NK activity** against K562, by BA11 before and after absorption by different cells, including the K562 target, was determined. RESULTS: R80K was detected on term placental syncytio and cytotrophoblast and on BeWo cells, by CELISA. BA11 suppressed NK lysis of K562 cell sin a dose-dependent manner. Absorption of the BA11 by BeWo and by cytotrophoblastic cells significantly decreased the NK-inhibitory activity. There was minimal absorption by K562 and BA11-pretreateed K562 cells remained susceptible to NK lysis. By contrast, BA11-pretreated peripheral blood cells lost all **NK activity**. CONCLUSIONS: The inhibition of NK killing of K562 cells by BA11 is more complex than simple masking of a trophoblast cell-associated molecule in K562 necessary for recognition in NK cells.

L26 ANSWER 17 OF 38 MEDLINE on STN DUPLICATE 7

96194234. PubMed ID: 8606382. Antimetastatic and antitumor activities of interleukin 10 in a murine model of breast cancer. Kundu N; Beaty T L; Jackson M J; Fulton A M. (Department of Pathology and the University of Maryland Cancer Center, Baltimore, 21201, USA.) Journal of the National Cancer Institute, (1996 Apr 17) 88 (8) 536-41. Journal code: 7503089. ISSN: 0027-8874. Pub. country: United States. Language: English.

AB BACKGROUND: Interleukin 10 (IL-10) is a potent immunoregulatory cytokine. It inhibits some cell functions, including T-helper (Th1) cell activity (i.e., interleukin 2 and interferon gamma production), and **stimulates** other functions such as a natural killer (**NK**) **activity**. In mice, IL-10 suppresses tumorigenicity in a xenograft system using a nonmetastasizing hamster cell line. PURPOSE: We evaluated the antitumor and antimetastatic properties of IL-10 in syngeneic immunocompetent and immunocompromised murine hosts. **METHODS**: Using the plasmids pBMGneo and pBMGneo.IL-10, we transfected the highly malignant murine mammary tumor cell lines 410.4 and 66.1 (transfectants designated as 410.4-IL10 and 66.1-IL10, respectively) to stably express IL-10 (2-100 U IL-10/2.5 x 10(5) cells per 48 hours). Tumorigenic and metastatic activities of the parent and transfected cells w ere measured in immunocompetent, syngeneic BALB/cByJ mice as well as in immunocompromised C.B-17/IcrCrl-SCID/Beige mice. RESULTS: Tumor growth was completely inhibited following inoculations of 5 x 10(6)410.4-IL10 cells in immunocompetent, syngeneic BALB/cByJ mice. This inoculum contains 100 times the minimum cell number required for 100% tumor incidence. In contrast, tumor growth following the inoculation of parental 410.4 or 410.4-neo cells was progressive, resulting in death of animals from pulmonary metastases at days 40-50 and transplantation. The tumorigenicity of 66.1-IL-10, compared with that of its parent cell line, was also significantly abrogated by IL-10 expression. Furthermore, in immunocompetent mice, the metastatic potential of both 410.4-IL10 and 66.1-IL10 was also completely inhibited. In immunocompromised C.B-17/IcrCrl-SCID/BR or C.B-17/IcrCrl-SCID/Beige mice, subcutaneous implants of 410.4-IL10 grew progressively, but growth was inhibited

significantly in comparison to that produced by the parental 410.4 or 410.4-neo cells. In spite of the more limited efficacy of IL-10 against tumor growth in immunocompromised mice, spontaneous metastasis of 410.4-IL10 cells in C.B-17/IcrCrl-SCID/BR mice was inhibited by 90%. When **NK activity** was suppressed by asialoGM1 ganglioside **antibody** in BALB/cByJ mice or in C.B-17/IcrCrl-SCID/Beige mice, the antimetastatic effect of IL-10 was lost. CONCLUSIONS: These data show for the first time that IL-10 is a potent antimetastatic agent that is effective in immunocompromised hosts. This effect thus appears to be relatively independent of T-cell function but is dependent on **NK activity**. In contrast, the inhibitory effect of IL-10 on tumorigenicity relies on T-cell function. IMPLICATIONS: Based on the recent observation of others that IL-10 has little toxicity when administered systemically to human volunteers and also on the findings of this study that it has antitumor and antimetastitic properties in mice, possible use of IL-10 in the treatment of human metastatic cancers deserves consideration.

L26 ANSWER 18 OF 38 MEDLINE on STN DUPLICATE 8

97067794. PubMed ID: 8911142. Cytotoxic activity against tumour cells mediated by intermediate TCR cells in the liver and spleen. Kawamura T; Kawachi Y; Moroda T; Weerasinghe A; Iiai T; Seki S; Tazawa Y; Takada G; Abo T. (Department of Pediatrics, Akita University School of Medicine, Japan.) Immunology, (1996 Sep) 89 (1) 68-75. Journal code: 0374672. ISSN: 0019-2805. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Morphological and phenotypic characterization in previous studies has indicated that intermediate (int) T-cell receptor (TCR) cells or T natural killer (TNK) cells may stand at an intermediate position between NK cells and high TCR cells of thymic origin in phylogenetic development. In this study, a functional study on cytotoxic activity against various tumour targets was performed in each purified subset. When a negative selection **method** entailing in vivo injection of anti-asialo GM, **antibody** or anti-interleukin (IL)-2R beta monoclonal **antibody** (mAb) was applied, IL-2R beta 1 CD3 NK cells were found to have the highest **NK activity** while IL-2R beta 1 int CD3 (or TCR) cells had a lower level of the **NK activity** . High CD3 cells (freshly isolated) did not have any such activity. Sorting experiments further revealed that the NK function mediated by int CD3 cells was augmented when they were exposed to anti-CD3 mAb. anti-TCR alpha beta, or anti-TCR-delta mAb. This phenomenon was not observed in NK cells and high CD3 cells. More importantly, when anti-CD3 mAb (or anti-TCR mAb) was added to the assay culture, int CD3 cells became cytotoxic against even NK-resistant tumour (Fc gamma R-. Fas+) targets. Liver mononuclear cells or int CD3 cells exposed to anti-CD3 mAb for 6 hr showed an elevated level of perforin in their cytoplasms. The present results suggest that int CD3 cells are usually non-cytotoxic against various tumours but become functional after being **stimulated** via the TCR CD3 complex.

L26 ANSWER 19 OF 38 MEDLINE on STN DUPLICATE 9

95385098. PubMed ID: 7544692. Mechanisms of enhancement of natural killer activity by an **antibody** to CD44: increase in conjugate formation and release of tumor necrosis factor alpha. Tan P H; Liu Y; Santos E B; Sandmaier B M. (Transplantation Biology Program, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA.) Cellular immunology, (1995 Sep) 164 (2) 255-64. Journal code: 1246405. ISSN: 0008-8749. Pub. country: United States. Language: English.

AB In this report, we found that an anti-CD44 mAb, S5, augmented conjugated formation between the effector cells (canine PBMC) and an NK-sensitive target cell line [canine thyroid adenoma carcinoma cell line (CTAC)], as determined by two different **methods**: conjugate enumeration using fluorescence microscopy and two-color flow cytometric analysis. However,

no increase in conjugate formation was seen when an NK-resistant target was used. This enhancement of initial conjugate formation could not be blocked using an **antibody** to CD18 (60.3), indicating that S5 possibly acted through another adhesive molecule(s). But the NK killing activity can be partially blocked by 60.3, indicating that this molecule may be important for later adhesive events. We also found that S5 **stimulated** the release of a heat labile cytotoxic factor from canine PBMC. This factor was found to lyse only TNF alpha-sensitive targets (CTAC and L929 cell lines) and caused apoptosis in the target cells. Its bioactivity was neutralized by polyclonal **antibody** to TNF alpha. All of these observations were consistent with the fact that the factor was TNF alpha. Our data suggested that the two mechanisms responsible for the enhancement in canine **NK activity** by an mAb to CD44 were an increase in conjugate formation and the release of TNF alpha.

L26 ANSWER 20 OF 38 MEDLINE on STN

95375966. PubMed ID: 7647961. Enhancement of immunostimulatory activity by dual substitution of C8-substituted guanine ribonucleosides: correlation with increased cytokine secretion. Pope B L; Chourmouzis E; Lee S; Goodman M G. (R. W. Johnson Pharmaceutical Research Institute, Don Mills, Ontario, Canada.) Journal of immunotherapy with emphasis on tumor immunology : official journal of the Society for Biological Therapy, (1995 Feb) 17 (2) 98-108. Journal code: 9418950. ISSN: 1067-5582. Pub. country: United States. Language: English.

AB Guanine ribonucleosides with single substitutions at the C8 position (monosubstituted) or with dual substitutions at the C8 and N7 positions (disubstituted) up-regulate a spectrum of immunologic responses, including cytolytic responses to tumor cells. The current studies were undertaken to determine the effects of dual substitution on a number of nucleoside-inducible immunological parameters. To do so, two monosubstituted analogues, 8-bromoguanosine and 8-mercaptoguanosine, were directly compared with two disubstituted analogues, 7-methyl-8-oxoguanosine and 7-allyl-8-oxoguanosine (loxoribine). All of the compounds enhance natural killer (**NK**) **activity**, lymphocyte proliferation, and **antibody** production in dose-dependent fashion. However, the potency and maximal activity of the disubstituted analogues are considerably greater than those of the monosubstituted analogues. Spleen cells **stimulated** for 48 h with the disubstituted compounds produce immunoreactive interleukin (IL) 1 alpha, IL-6, tumor necrosis factor-alpha (TNF alpha), and interferon-gamma (IFN gamma). Monosubstituted analogues induce lower quantities of IL-6, TNF alpha, and IFN gamma and fail to induce detectable levels of IL-1 alpha. Total IFN activity, assessed by viral inhibition assay, is also lower for the monosubstituted analogues. Augmentation of **antibody** secretion by B cells is diminished for neither mono- nor disubstituted compounds upon incubation with anti-cytokine **antibodies**. In contrast, anti-IFN alpha beta markedly reduces the effects of monosubstituted analogues on **NK activity** but has less marked effects on NK induction by the disubstituted compounds. A similar pattern of differences is seen for lymphocyte proliferation. Thus, although the analogues induce synthesis of several cytokines, to date only IFN alpha beta appears directly involved in enhancement of **NK activity** and lymphocyte proliferation. The present data do not, however, exclude the existence of an autocrine **stimulatory** mechanism not susceptible to inhibition by anti-cytokine **antibodies**.

L26 ANSWER 21 OF 38 CAPLUS COPYRIGHT 2004 ACS on STN

1995:18495 Document No. 122:29615 Structure and physicochemical properties of purified human leukocyte interferon (FPI-31). Uemura, Hidetoshi; Negoro, Kenji; Ueda, Yasuhiko; Chisaka, Takeshi; Shirono, Hiroyuki; Kono,

Keiko; Yamamoto, Yoshiki; Son, Karei; Doi, Teruko; et al. (Res. Dev. Cent., Fuso Pharm. Ind., Ltd., Osaka, 536, Japan). Iyakuhin Kenkyu, 25(3), 171-85 (Japanese) 1994. CODEN: IYKEDH. ISSN: 0287-0894.

AB The structure and physicochem. properties of human leukocyte interferon (FPI-31) were studied. FPI-31 was purified from the supernatant of human leukocyte suspension **stimulated** with Sendai virus, by **antibody** affinity chromatog. and a modified Cantell **method**. Seventeen subtypes were found in the FPI-31 preparation by reverse-phase HPLC, SDS-PAGE and N-terminal amino acid sequence anal. In addition, N-asparagine-linked sugar chain was detected in 3 subtypes. Mol. wts. and pIs were distributed from 16,000 to 24,000 daltons and from pH 5.0 to pH 7.1, resp. The antigenicity of FPI-31 was identified as that of interferon (IFN) α by neutralization of its antiviral activity with specific **antibody**. Cytokines such as TNF, IL-1, IL-2, GM-CSF or G-CSF were not detected in FPI-31 by ELISA or biol. assay. FPI-31 was sensitive to heat and light exposure but was stable over a wide range of pH and to treatment with various enzymes except proteases. The degrees of augmentation of **NK activity** and induction of 2'-5' oligo-adenylate synthetase activity and the subtype composition were well conserved over 5 lots of FPI-31.

L26 ANSWER 22 OF 38 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

91:639395 The Genuine Article (R) Number: GP791. IFN-BETA INDUCED BIOCHEMICAL AND IMMUNOLOGICAL MODIFICATIONS IN HAIRY-CELL LEUKEMIA PATIENTS. LIBERATI A M (Reprint); SCHIPPA M; PORTUESI M G; PROIETTI M G; DEANGELIS V; FERRAJOLI A; CINIERI S; DICLEMENTE F; PALMISANO L; BERRUTO P. UNIV PERUGIA, MONTELUCE POLICLIN, MED CLIN 1, I-06100 PERUGIA, ITALY (Reprint); IND FARMACEUT SERONO, ROME, ITALY; OSPED CIVILE, SERV MED NUCL, TERNI, ITALY; IST RIC BIOMED ANTOINE MARXER SPA, IVRED, ITALY. HAEMATOLOGICA (1991) Vol. 76, No. 5, pp. 375-382. Pub. country: ITALY. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background. Although IFN-beta is 30-40% homologous with IFN-alpha, its intrinsic biological properties are not identical. Compared with IFN-alpha, IFN-beta exerts greater in vitro antiproliferative activity on many cell lines, **stimulates** peripheral blood stem cells of hairy-cell leukemia (HCL) patients to differentiate to erythroid burst forming cells, has higher specific type I IFN receptor affinity and modulates the expression of class II histocompatibility antigens. IFN-beta would, therefore, be expected to have a greater, or at least similar, antitumor activity as that of the various types of IFN-alpha.

Methods. We have treated 12 patients affected by HCL with IFN-beta and have investigated the biological and immunological changes induced by such treatment.

Results. A rise in beta-2-microglobulin and neopterin values throughout IFN-beta therapy was documented in most patients. An increase in **NK activity** was observed only in clinical responders whose CD57+/CD16+ cell ratio dropped below baseline. There was also a modulation in IFN-gamma synthesis that was dependent on baseline levels and in line with the clinical response. IFN-beta provoked a reduction in CD3+ and CD4+ cell subsets in patients with WBC greater-than-or-equal-to 10.0 x 10(9)/l and greater-than-or-equal-to, 50% circulating HCs, an expansion in absolute number of CD3+ and CD8+ cell fractions and a slight rise in the absolute values of CD2+ and CD4+ cell subpopulations in patients with WBC less-than-or-equal-to 5.0 x 10(9)/l and less-than-or-equal-to 50% circulating HCs. There was no correlation between either the IFN-beta induced increase in beta-2-M or Np levels and clinical response. Most immunological parameters improved or normalized later during the course of IFN-beta treatment, when pathological-hematological signs of disease remission were already evident.

Conclusions. The relevance of the IFN-beta induced changes as well as that of the IFN-alpha induced biological effects in the clinical control

of HCL remain unclear.

L26 ANSWER 23 OF 38 MEDLINE on STN DUPLICATE 10
90117497. PubMed ID: 2136961. Effects of ozone, hexachlorobenzene, and bis(tri-n-butyltin)oxide on natural killer activity in the rat lung. Van Loveren H; Krajnc E I; Rombout P J; Blommaert F A; Vos J G. (National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.) Toxicology and applied pharmacology, (1990 Jan) 102 (1) 21-33. Journal code: 0416575. ISSN: 0041-008X. Pub. country: United States. Language: English.

AB The respiratory tract is a major route of exposure to noxious agents as well as pathogens such as viruses. Natural killer (**NK**) **activity** is an important first line of defense to virally infected cells as well as certain neoplasms; therefore, testing the effects of exposure to toxic compounds on this activity is important in understanding the immunotoxic potential of the compound. Lymphoid cell suspensions, obtained after enzymatic dispersion of rat lungs and purification over nylon wool columns, showed in vitro natural killer activity toward YAC lymphoma cells. Validation of the test with well-known **NK activity stimulators** such as Bacillus Calmette-Guerin (BCG), interleukin-2 (IL-2), interferon (IFN), and inhibitors like anti-asialo-GM1 (ganglio-n-tetrasylceramide) **antibody** confirmed the reliability of the test as an assay for detecting **NK activity** in rat lungs. Using this assay, we studied the effects of exposure to ozone (O3), hexachlorobenzene (HCB), and bis(tri-n-butyltin)oxide (TBTO) on **NK activity** in rat lung. Inhalation exposure to O3 for 7 days at 0.4 and 0.8 mg/m3 resulted in **stimulation**, and exposure at 1.6 mg O3/m3 resulted in suppression of **NK activity**. Oral exposure to HCB in concentrations of 150 and 450 mg/kg food for 6 weeks suppressed **NK activity** in rat lungs in a dose-related manner. This was also true for 6 weeks of oral exposure of rats to 20 and 80 mg TBTO/kg food, but to a lesser extent. In summary, we have developed and validated a **method** to measure the effects of (toxic) substances on **NK activity** in rat lung.

L26 ANSWER 24 OF 38 MEDLINE on STN DUPLICATE 11
89266658. PubMed ID: 2786245. Specific depletion of mature T lymphocytes from human bone marrow. Geisler C; Moller J; Plesner T; Dickmeiss E; Pallesen G; Larsen J K; Jacobsen N; Svejgaard A. (Department of Clinical Immunology, Finsen Laboratory, Copenhagen, Denmark.) Scandinavian journal of immunology, (1989 May) 29 (5) 617-25. Journal code: 0323767. ISSN: 0300-9475. Pub. country: ENGLAND: United Kingdom. Language: English.

AB An effective **method** for specific depletion of mature T lymphocytes from human bone marrow mononuclear cells (BMMC) with preservation of prethymic T cells and natural killer (NK) cells is presented. The BMMC were incubated with F101.01, a monoclonal **antibody** recognizing an epitope of the T-cell receptor-CD3 complex, and subsequently with immunomagnetic beads. Flow cytometric analysis demonstrated that mature T cells were efficiently depleted and that NK cells and prethymic T cells were preserved in the BMMC. Furthermore, T cell-mediated immune reaction as measured by thymidine incorporation after **stimulation** with phytohaemagglutinin was abolished, whereas **NK activity** as measured by 51Cr release using K562 as target cells was preserved. Recovery of colony-forming units of granulocyte-macrophages was 60-70%.

L26 ANSWER 25 OF 38 MEDLINE on STN DUPLICATE 12
88168656. PubMed ID: 3258280. Unfractionated spleen cells but not natural killer (NK) cells from RFM donors prevent the progression of host-versus-graft disease in murine RFM/(T6 x RFM)F1 chimeras. Hard R C; Patel M R; Keller L M; Linna T J. (Department of Pathology, Medical

College of Virginia/VCU, Richmond 23298-0662.) Immunology, (1988 Mar) 63 (3) 457-64. Journal code: 0374672. ISSN: 0019-2805. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Host-versus-graft (HVG) syndrome is the fatal allogenic disease which develops in susceptible strains of inbred mice following their perinatal inoculation with related F1 hybrid spleen cells. Deaths are caused by pathogenic immune complexes. It is thought that the **antibody** components of these complexes are produced by F1 donor B cells **stimulated** by the allogenic HVG reaction. To complement previous work that showed that lethal disease could be prevented if the HVG response was suppressed, the present studies tested whether or not it could also be prevented by augmenting HVG reactivity with the adoptive transfer of spleen cells syngenic with the host. The data show that unfractionated RFM spleen cells given on Days 13-14 prevented lethal disease in 86% of RFM/(T6 x RFM)F1 chimeras. Successful therapy was associated with the suppression of formation of nephropathic-immune complexes, and with the rejection of F1 donor cells or their gradual replacement by host cells. RFM spleen cells enriched for **NK activity** by a new improved **method** not only failed to prevent HVG disease but appeared to exacerbate it. This was also true of spleen cells that had been activated in vitro for 3 days with IL-2, a procedure that greatly enhanced their cytolytic activity against YAC-1 targets. It is suggested that therapy with NK cells failed, even after IL-2 activation, because they had no effect on proliferating and **antibody**-forming F1 donor cells that had engrafted in large numbers in the lymph nodes of the RFM hosts.

L26 ANSWER 26 OF 38 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1988:266947 Document No.: PREV198886006191; BA86:6191. SUPPRESSION OF MYELOID HEMATOPOIESIS BY LEUKEMIA NATURAL KILLER CELLS BEARING A LEU-11-POSITIVE LEU-7-NEGATIVE PHENOTYPE INTERFERON-GAMMA AS A POSSIBLE MEDIATOR. KOIZUMI S [Reprint author]; TACHINAMI T; SAIKAWA Y; TANIGUCHI N. DEP PEDIATRICS, KANAZAWA UNIV SCH MED, 13-1 TAKARAMACHI, KANAZAWA, ISHIKAWA 920, JPN. Acta Haematologica Japonica, (1988) Vol. 51, No. 1, pp. 81-93.
CODEN: NKGZAE. ISSN: 0001-5806. Language: ENGLISH.

AB Natural killer (NK) cells, morphologicaly indistinguishable from large granular lymphocytes (LGLs) are thought to have an important role in the regulation of hematopoiesis. A clonal leukemic population of NK cells was isolated from a 14-year-old girl. They had a Leu-11+, Leu-7- phenotype. Since the **NK activity** was low but was remarkably augmented by **stimulation** with recombinant human interleukin 2 (rIL-2), but not interferon-γ (IFN-γ), this NK subset seems to belong to a premature stage in the maturation of NK cells. Because of severe neutropenia throughout the clinical course, we investigated the effects of this NK subpopulation on the in vitro growth of granulocyte-macrophage colony forming cells (GM-CFC). When this NK subset in the presence of rIL-2 was added to the GM-CFC assay system employing normal bone marrow cells, a remarkable suppression of GM-CFC was observed. Culture supernatants from the rIL-2-**stimulated** NK cells, which had a high level of IFN-γ, also exhibited significant suppressive activity. Treatment of the supernatants with a specific **antibody** against IFN-γ abrogated the inhibitory effect on GM-CFC. These data indicate that this unique subset of premature NK cells in the presence of rIL-2 produces IFN-γ, which seems to be involved in the suppression of myelopoietic progenitor cells.

L26 ANSWER 27 OF 38 MEDLINE on STN DUPLICATE 13
88034398. PubMed ID: 2444651. Natural killer cell inhibition of young spherules and endospores of Coccidioides immitis. Petkus A F; Baum L L. (Department of Microbiology and Immunology, Chicago Medical School, IL 60064.) Journal of immunology (Baltimore, Md. : 1950), (1987 Nov 1) 139 (9) 3107-11. Journal code: 2985117R. ISSN: 0022-1767. Pub. country:

United States. Language: English.

AB The recent development of a **method** for culturing the parasitic form of Coccidioides immitis by using conditions compatible with the growth of lymphoid cells has enabled us to investigate the role of natural killer (NK) cells in defense against this pathogenic fungus. Pure cultures of spherules and endospores were grown in RPMI 1640 which contained 10% calf serum. Single cell suspensions of young spherules and endospores were incubated in the presence of freshly isolated human peripheral blood lymphocytes (PBL). After a 4-hr incubation, the colony-forming ability of the fungus was significantly reduced. Leu-11 is a monoclonal **antibody** that binds to the Fc receptor of NK cells. When PBL were incubated in the presence of this monoclonal **antibody** and complement, the colony-forming ability of C. immitis was not reduced, indicating that the effector cell involved in reduction of colony-forming units is also recognized by the Leu-11 monoclonal **antibody**. Classical **NK activity** can be enhanced by preincubation with interferon; the inhibitory activity of the PBL which are responsible for the reduction in colony-forming units of C. immitis is similarly enhanced by pretreatment with interferon. When PBL are incubated in the presence of young spherules and endospores for 24 hr, the cellfree supernatants will kill U937 target cells. In addition to **stimulating** the release of NK cytotoxic factor, C. immitis is susceptible to inactivation when incubated in the presence of factors released by PBL which have been incubated in the presence of either K562 or C. immitis. Other evidence reported by this laboratory demonstrates that C-reactive protein is present on the surface of NK cells and that **antibody** to this molecule blocks NK-mediated killing of standard tumor cell targets. Pretreatment with anti-C-reactive protein also blocks the ability of PBL to inhibit the colony-forming capacity of this fungus. These data suggest that the cell that inhibits the in vitro growth of the pathogenic fungus, C. immitis, is an NK cell.

L26 ANSWER 28 OF 38 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

1988:28573 Document No.: PREV198885016298; BA85:16298. HUMAN CD3+4-8-WT31-T LYMPHOCYTE POPULATIONS EXPRESSING THE PUTATIVE T CELL RECEPTOR GAMMA-GENE PRODUCT A LIMITING DILUTION AND CLONAL ANALYSIS. MORETTA L [Reprint author]; PENDE D; BOTTINO C; MIGONE N; CICCONE E; FERRINI S; MINGARI M C; MORETTA A. IST NAZIONALE PER LA RICERCA SUL CANCRO, I-16132 GENOVA, ITALY. European Journal of Immunology, (1987) Vol. 17, No. 9, pp. 1229-1234. CODEN: EJIMAF. ISSN: 0014-2980. Language: ENGLISH.

AB The small peripheral blood CD3+ T cell population lacking both CD4 and CD8 surface antigens has been analyzed in the present study. Enriched CD3+4-8- populations were obtained by depletion with anti-CD4 or anti-CD8 monoclonal **antibodies** (mAb) and complement. The resulting populations contained > 99% CD2+ cells, whereas CD3+ represented 50%. Virtually all of the cells were CD4-8- and did not react with the WT31 mAb, specific for a framework determinant of the α/β T cell receptor (TCR). In order to analyze the molecular nature of CD3-associated molecules in CD3+WT31- populations, cells were **stimulated** with 0.5% phytohemagglutinin (PHA) for 24 h and expanded for an additional 7-14 days in interleukin 2 (IL-2). The resulting cells were > 95% CD3+ and expressed neither CD4/CD8 nor WT31 antigen. Cell surface iodination followed by cross-linking and immunoprecipitation with anti-CD3 mAb showed that CD3-associated molecules consisted of a major 45-kDa band and a minor band of 43 kDa. Thus, whereas CD3-associated molecules isolated from polyclonal CD3+WT31+ populations (expanded in IL 2 under the same culture conditions) appeared as diffuse bands, CD3-associated molecules isolated from CD3+WT31- populations displayed a homogeneous molecular mass. Northern blot analysis revealed the presence of mRNA for the TCR γ chain whereas the mRNA for the α chain was mostly represented by a truncated (1.2 kb) form. Also small amounts of a nonproductive mRNA for the β chain

were detected. Freshly isolated CD3+WT31--enriched populations proliferated in response to PHA and concanavalin A, moreover, IL 2 was detected in the culture supernatants after cell **stimulation**. By applying culture conditions which allow virtually all T cells to undergo clonal expansion, approximately 1/3 CD3+WT31- were clonogenic. In addition, the large majority of proliferating microcultures lysed the K562 cell line and about half the natural killer (NK)-resistant fresh melanoma target cells. A large number of clones derived from CD3+WT31- enriched populations by limiting dilution has been further analyzed. More than 955 of the clones were CD3+4-8-WT31-; 12/15 clones analyzed in more detail displayed **NK activity** and 6/15 lysed melanoma cells; in addition, all lysed P815 target cells in the presence of PHA, thus indicating that all the clonogenic CD3+WT31- cells have a cytolytic potential.

L26 ANSWER 29 OF 38 MEDLINE on STN

86087234. PubMed ID: 3079800. Organ-associated macrophage precursor activity: isolation of candidacidal and tumoricidal effectors from the spleens of cyclophosphamide-treated mice. Baccarini M; Bistoni F; Lohmann-Matthes M L. Journal of immunology (Baltimore, Md. : 1950), (1986 Feb 1) 136 (3) 837-43. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB We recently reported the modulating effects of a single injection of the anti-neoplastic drug cyclophosphamide (Cy; 150 mg/kg i.p.) on in vivo resistance against the experimental Candida albicans infection. Increased resistance to microbial challenge occurred 12 to 18 days after treatment. We now show that the increased resistance is paralleled by the appearance of potent nonadherent nonphagocytic effectors in the spleen (day 12) that are capable both of candidacidal activity and natural killer (**NK**) **activity** against YAC-1 cells. The cells mediating the two reactivities have a low buoyant density, a strong proliferating activity in response to the macrophage colony **stimulating** factor (CSF-1), and are unable to kill the NK-insensitive lines EL-4 and P815. A clear cut isolation of macrophage precursor cells from this Percoll low density fraction has been performed in an indirect rosette assay on the basis of their positivity for the surface markers recognized by the highly specific rat-anti-mouse macrophages, monoclonal **antibodies** M143 and F4/80. We obtained an extremely homogeneous population of cells in the early stage of macrophage differentiation that is responsible for all the candidacidal activity and for a major part of the **NK** **activity** observed in the spleen of Cy-treated mice, and which is extremely sensitive to CSF-1 induction.

L26 ANSWER 30 OF 38 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

1987:85628 Document No.: PREV198783044206; BA83:44206. ESTABLISHMENT OF TAC-NEGATIVE INTERLEUKIN 2-DEPENDENT CYTOTOXIC CELL LINES FROM LARGE GRANULAR LYMPHOCYTES OF PATIENTS WITH EXPANDED LARGE GRANULAR LYMPHOCYTE POPULATIONS. PISTOIA V [Reprint author]; CARROLL A J; PRASTHOFER E F; TILDEN A B; ZUCKERMAN K S; FERRARINI M; GROSSI C E. ISTITUTO DI ONCOLOGIA CLINICA E SPERIMENTALE, UNIV DI GENOVA, ISTITUTO NAZIONALE PER LA RICERCA SUL CANCRO, GENOVA, ITALY. Journal of Clinical Immunology, (1986) Vol. 6, No. 6, pp. 457-466.
CODEN: JCIMDO. ISSN: 0271-9142. Language: ENGLISH.

AB Cell lines were established from purified large granular lymphocytes (LGL) isolated from the peripheral blood of seven patients with phenotypically homogeneous LGL expansions. LGL were **stimulated** with phytohemagglutinin (PHA) or recombinant interleukin-2 (rIL-2) and further expanded in vitro in IL-2-containing media. The surface phenotype of LGL, as assessed by monoclonal **antibody** staining, was T3+ T8+ in five patients, T3- T8- in one, and T3+ T8+ in another patient. The cells also expressed Leu 7, Leu 11, and/or OKM 1 markers in various proportions and were identifiable as LGL by their morphological and cytochemical features.

The original surface phenotype of the unstimulated LGL was retained in the IL-2-dependent cell lines from each individual patient, i.e., T3+ T8+ cells originated T3+ T8+ cell lines and T3- T8- cells originated T3- T8- cell lines. Other markers, such as Leu 11 and OKM 1, were generally lost in culture. LGL proliferated in response to rIL-2 but did not express detectable IL-2 receptors, even after prolonged periods of culture. All cell lines from each individual patient had the same surface phenotype, and within the single lines, all of the cells expressed the same markers. Cell lines from two patients consistently displayed chromosomal abnormalities. Although different in the two patients, the abnormalities were identical in all of the lines from the same patient and detectable in most of the cells examined, suggesting a clonal origin for the abnormally expanded LGL populations. Freshly isolated LGL did not exert **NK activity**. However, the IL-2-dependent LGL lines acquired the ability to kill K562 target cells and to produce gamma interferon (γ-IFN). No direct correlation was observed between these two properties.

L26 ANSWER 31 OF 38 MEDLINE on STN

86133203. PubMed ID: 2936447. Systemic administration of autologous, alloactivated helper-enriched lymphocytes to patients with metastatic melanoma of the lung. A phase I study. Balsari A; Marolda R; Gambacorti-Passerini C; Sciorelli G; Tona G; Cosulich E; Taramelli D; Fossati G; Parmiani G; Cascinelli N. Cancer immunology, immunotherapy : CII, (1986) 21 (2) 148-55. Journal code: 8605732. ISSN: 0340-7004. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB A phase I study was carried out to test the feasibility and toxicity of infusing large numbers of autologous, alloactivated helper lymphocytes into patients with metastatic melanoma. Patient peripheral blood lymphocytes (Pt-PBL) obtained by lymphopheresis and expressing the helper phenotype BT5/9 were separated and **stimulated** for 48 or 72 h with a pool of PBL from four to six healthy donors. Patients were then infused with such activated lymphocytes over a 2-3 h period. A total of 4 phereses and infusions (2/week for 2 weeks) were carried out for each cycle in each patient. Of the five patients treated, two received a second round of infusions. Infusion of autologous PBL **stimulated** in vitro for 48 h caused chills, fever, headache, and increased blood pressure. All symptoms disappeared in 2-3 h and were easily controlled by appropriate therapy. When lymphocytes were given after 72 h of allostimulation, no or very mild toxicity was observed. Serum chemistry, coagulation, autoimmunity, and urine analysis showed no gross abnormalities during therapy or follow-up of the patients. Immunological parameters (OKT4/OKT8 ratio, **NK activity** and cytotoxic T cell activity to autologous melanoma) were evaluated before starting the therapy, during its course and during the 3 to 6 months follow-up. The OKT4/OKT8 ratio increased significantly but transiently soon after the first course of infusions in one of the two patients tested. **NK activity** increased after 75-100 days in the three patients tested and in one of them it was high even after 180 days. No correlation between **NK activity** and prognosis was apparent.
Cytotoxicity to autologous tumor was assessed in two patients, only of one of whom exhibited an increased activity from 75 to 180 days, which was associated with a prognosis better than that of the negative patient. Five patients were treated: two had progressive disease, two had stable disease for 5 and 6 months, respectively. In the first of these patients, a new cycle of lymphocyte infusions was carried out which caused a measurable reduction of lung tumor nodules whose growth, however, resumed 4 months later. This patient died 14 months after the onset of therapy. The fifth patient had a partial regression of pulmonary and intracranial metastases after therapy, but eventually died 3 months later. These results indicate that infusion of a high numbers of autologous, allostimulated helper PBL is a feasible and safe procedure, which could

therefore be used in future studies of adoptive immunotherapy of cancer.

L26 ANSWER 32 OF 38 MEDLINE on STN DUPLICATE 14
85132637. PubMed ID: 2579129. Suppression of alloimmune cytotoxic T lymphocyte (CTL) generation by depletion of NK cells and restoration by interferon and/or interleukin 2. Suzuki R; Suzuki S; Ebina N; Kumagai K. Journal of immunology (Baltimore, Md. : 1950), (1985 Apr) 134 (4) 2139-48. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB By using rabbit antiserum to a glycolipid, ganglio-n-tetraosylceramide (ASGM1), the accessory effect of natural killer (NK) cells on the generation of alloimmune CTL in mice was investigated. When normal C3H/He mice were immunized with C57BL/6 or BALB/c spleen cells, they generated alloimmune CTL with a surface marker phenotype of Thy-1+ Lyt-1-2+ ASGM1-, preceded by early augmentation of cytotoxic activity of NK cells with a Thy-1-Lyt-1-2-ASGM1+ phenotype. Administration of anti-ASGM1 (10 microliters) in mice resulted in a complete depletion of **NK activity** and ASGM1+ cells in the spleen even 1 day after injection, but no changes in the proportions of T (Thy-1+) cells and their Lyt-1 and Lyt-2 subsets as revealed by an immunofluorescence analyzer (FACS) and phagocytic cells. When these anti-ASGM1-treated mice were immunized with allogeneic cells, they showed neither augmented **NK activity** nor generation of alloimmune CTL, and spleen cells isolated from these anti-ASGM1-treated mice produced no CTL response to alloimmunization in vitro. Normal spleen cells treated with the antiserum and complement in vitro also showed a complete NK depletion without any deterioration of T cells and their Lyt-1 and Lyt-2 subsets, and when **stimulated** with allogeneic cells they generated no CTL. Spleen NK (ASGM1+) cells were purified by Percoll-gradient centrifugations followed by complement-dependent killing of T cells with the use of anti-Thy-1 monoclonal **antibody**, and were further purified by panning **methods** with anti-ASGM1, giving a preparation consisting of greater than 90% ASGM1+, Ly-5+ cells, and less than 0.5% of Thy-1+, Lyt-1+, and Lyt-2+ cells. These purified ASGM1+ Thy-1- cells alone generated no alloimmune CTL in response to alloantigens, suggesting that ASGM1+ NK cells contained no precursors of alloimmune CTL. When added into NK-depleted spleen cells, they restored the normal alloimmune CTL response of the spleen cells, indicating that ASGM1+ fractions contained cells to provide an accessory function for CTL generation. Lyt-1+ cells purified by panning **methods** did not restore the CTL response of NK-depleted spleen cells. These results indicate that ASGM1+ NK cells, but not Lyt-1+ helper T cells contaminating ASGM1+ fractions at undetectable levels, are responsible for the accessory function. When these purified ASGM1+ Thy-1- cells were **stimulated** with allogeneic cells, they produced IL 2 and IFN.(ABSTRACT TRUNCATED AT 400 WORDS)

L26 ANSWER 33 OF 38 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1985:398140 Document No.: PREV198580068132; BA80:68132. COMPARISON OF THE CELLULAR CYTOTOXIC ACTIVITIES OF COLOSTRAL LYMPHOCYTES AND MATERNAL PERIPHERAL BLOOD LYMPHOCYTES. NAIR M P N [Reprint author]; SCHWARTZ S A; SLADE H B; JOHNSON M Z; QUEBBEMAN J F; BEER A E. DEP PEDIATR, UNIV MICHIGAN, ANN ARBOR, MI 48109, USA. Journal of Reproductive Immunology, (1985) Vol. 7, No. 3, pp. 199-214.
CODEN: JRIMDR. ISSN: 0165-0378. Language: ENGLISH.

AB Colostral lymphocytes (CL) from mothers 2-4 days post-partum and autologous maternal peripheral blood lymphocytes (PBL) were investigated for natural killer (NK) and **antibody**-dependent cellular cytotoxic (ADCC) activities, target binding ability, interferon (IFN)- and interleukin 2 (IL2)-induced augmentation of **NK activity**, lectin-dependent cellular cytotoxicity (LDCC), and the ability of culture-derived soluble suppressor factor(s) to inhibit the **NK**

activity of normal allogeneic lymphocytes. CL depleted of adherent cells and Percoll-separated NK-enriched subpopulations of CL demonstrated significantly lower NK and ADCC activities compared to autologous PBL. The target binding ability of CL was comparable to autologous PBL. Although the residual **NK activity** of CL was augmented by IFN and IL2, the activity was not enhanced to the same level shown by autologous PBL. CL also demonstrated a significant enhancement of LDCC activity, although the activity was not **stimulated** to the levels shown by PBL. Culture supernates of CL manifested greater suppression of the NK ability of allogeneic PBL than culture supernates produced by autologous PBL. Evidently, differential partitioning of lymphocyte subpopulations occurs between colostrum and peripheral blood.

L26 ANSWER 34 OF 38 MEDLINE on STN DUPLICATE 15
85201756. PubMed ID: 2581709. Recombinant interleukin 2 rapidly augments human natural killer cell activity. Kabelitz D; Kirchner H; Armerding D; Wagner H. Cellular immunology, (1985 Jun) 93 (1) 38-45. Journal code: 1246405. ISSN: 0008-8749. Pub. country: United States. Language: English.
AB Recombinant human interleukin 2 (r-IL-2) rapidly **stimulated** human natural killer cell activity in vitro. Augmentation of **NK activity** occurred within 1 hr of preincubation with r-IL-2. Responsive killer cells were typical NK cells as shown by cell fractionation procedures. These included Percoll density gradient separation and depletion of OKT3+ T cells by an indirect rosetting **method**. Analysis with a panel of polyclonal and monoclonal **antibodies** against alpha and gamma interferon revealed that this early enhancement of **NK activity** by r-IL-2 was independent of the production of both types of interferon.

L26 ANSWER 35 OF 38 MEDLINE on STN
84191498. PubMed ID: 6609314. Human large granular lymphocytes are potent producers of interleukin-1. Scala G; Allavena P; Djeu J Y; Kasahara T; Ortaldo J R; Herberman R B; Oppenheim J J. Nature, (1984 May 3-9) 309 (5963) 56-9. Journal code: 0410462. ISSN: 0028-0836. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Natural killer (**NK**) **activity** against tumour and virus-infected target cells is shown by a subpopulation of peripheral blood mononuclear leukocytes with the morphological features of large granular lymphocytes (LGL). The lineage of human LGL is still controversial, as they display surface markers of both T lymphocytes and myelomonocytic cells. LGL have recently been reported to produce lymphokines such as interleukin-2 (IL-2) and alpha- as well as gamma-interferons, functions associated mainly with T cells. To determine whether cytokines associated with other cell lineages are also produced by LGL, we examined whether they might produce a myelomonocyte -associated cytokine such as interleukin-1 (IL-1). IL-1 is a 12-18,000 molecular weight (MW) lymphokine produced by a variety of cell types such as monocytes, keratinocytes and a human dendritic cell line, which plays a crucial role in immunoregulation and inflammation. Moreover, IL-1 has recently been reported to act synergistically with IL-2 and interferons in boosting LGL-mediated **NK activity**. We now show that a subset of highly purified human LGL with **NK activity** can be **stimulated** to secrete a soluble factor with the biochemical and biological characteristics of human IL-1.

L26 ANSWER 36 OF 38 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1984:186398 Document No.: PREV198477019382; BA77:19382. NATURAL KILLER CELLS AS A RESPONDER TO INTERLEUKIN 2 1. PROLIFERATIVE RESPONSE AND ESTABLISHMENT OF CLONED CELLS. SUZUKI R [Reprint author]; HANDA K; ITOH K; KUMAGAI K. DEP MICROBIOL, TOHOKU UNIV SCH DENTISTRY, SENDAI, JAPAN. Journal of Immunology, (1983) Vol. 130, No. 2, pp. 981-987.

CODEN: JOIMA3. ISSN: 0022-1767. Language: ENGLISH.

AB The proliferative response of murine natural killer (NK) cells to a T cell growth factor, interleukin 2 (IL 2) was investigated by using the NK-enriched, low-density fractions (F · 1) and NK-depleted, T cell-enriched and high-density fractions (F · 3) of normal spleen cells after Percoll-discontinuous density gradient centrifugation. When F · 1 and F · 3 cells were cultivated with IL 2-containing medium without addition of any other factors, like lectins, feeder layer or macrophage products, F · 1 but not F · 3 cells showed an IL 2-dependent proliferative response. Growing populations showed a potent **NK activity** and consisted largely of cells with a morphology of large granular lymphocyte (LGL) and a surface marker profile characteristic for murine NK cells. By limiting dilution in the liquid culture and colony formation in the soft agar medium, F · 1 but not F · 3 cells showed a high frequency of clonal replication in IL 2. Almost all growing colonies in either medium showed a typical morphology of LGL. F · 1 cells treated with anti-asialo GM1 **antibody** and C [complement], from which NK cells and cytolytic activity were almost completely abrogated and for which T cells were enriched, conversely formed a few IL 2-dependent colonies. From the colonies in soft agar, 11 clones, all of which showed a typical morphology (LGL), a typical surface profile with the exception of lacking IgG-Fc receptors for NK cells (Thy-1±, Ly-5+, ASGM1+, Lyt-1-, Lyt-2-, FcγR-), and a cytotoxic activity of activated nature, were established and maintained for a period of longer than 1 yr in the medium containing IL 2 alone. A major population in the native spleens of normal mice that respond directly to IL 2 and clonally replicate without other **stimulating** factors may be NK cells.

L26 ANSWER 37 OF 38 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

1984:250882 Document No.: PREV198477083866; BA77:83866. INDUCTION OF PROLIFERATION AND NATURAL KILLER ACTIVITY IN HUMAN LYMPHOCYTES BY MATURE MYELO MONOCYTIC CELLS EVIDENCE FOR AN HLA-DR INDEPENDENT MIXED LYMPHOCYTE REACTION **STIMULATORY** ABILITY OF TERMINALLY DIFFERENTIATED NONLYMPHOID LEUKEMIC CELL LINES AND OF NORMAL PERIPHERAL BLOOD GRANULOCYTES. SANTOLI D [Reprint author]; FRANCIS M K; MATERA L; FERRERO D. WISTAR INST, 36TH AT SPRUCE ST, PHILADELPHIA, PA 19104, USA. Journal of Immunology, (1983) Vol. 131, No. 2, pp. 736-742.

CODEN: JOIMA3. ISSN: 0022-1767. Language: ENGLISH.

AB Three human myeloid leukemic cell lines (HL60, KG1 and ML3) and 1 histiocytic lymphoma line (U937) were induced to differentiate terminally to mature myelomonocytic cells with either 12-O-tetradecanoylphorbol-13-acetate (TPA) or lymphocyte-conditioned medium (LCM), which contains differentiation-inducing factors. HL60 cells were also forced to differentiate along the myeloid series with retinoic acid (RA) or dimethyl sulfoxide (DMSO). The striking morphologic changes and the expression of differentiated markers on the induced cells (whether macrophage- or granulocyte-like) were always associated with an acquired or dramatically increased ability to **stimulate** proliferation and natural killer cell (**NK**) **activity** in human lymphocytes. Like HL60 cells after granulocytic differentiation, granulocytes freshly separated from the peripheral blood of healthy donors were also strong inducers of mixed lymphocyte reaction (MLR) responses. Analysis of the expression of HLA-DR antigens on the surface of undifferentiated and mature cells with 2 monoclonal **antibodies** directed against HLA-DR monomorphic determinants, indicated that: upon differentiation induced with RA, DMSO and TPA the cells never acquired surface DR antigens; and normal peripheral blood granulocytes lacked these antigens. Treatment with LCM always resulted in the expression of high levels of DR antigens on the differentiated macrophage-like cells. Apparently, all mature myelomonocytic cells, either freshly separated from peripheral blood or obtained after forced in vitro differentiation of leukemic cells, express

MLR **stimulatory** antigens that appear to be unrelated to DR determinants. The possibilities discussed are that such antigens are associated with other molecules encoded by the D region or with new surface structures unrelated to Ia but dependent on the stage of differentiation.

L26 ANSWER 38 OF 38 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 16

81019224 EMBASE Document No.: 1981019224. Modulation of natural cytotoxicity by alloantibodies. III. Augmentation of natural killer activity by alloantisera directed against K, D, and I regions of the murine H-2 complex. Saxena R.K.; Saxena Q.B.; Adler W.H.. Clin. Physiol. Branch, Gerontology Res. Cent., Nat. Inst. Aging, NIH, Baltimore Md. 21224, United States. Cellular Immunology 56/1 (89-98) 1980.
CODEN: CLIMB8. Pub. Country: United States. Language: English.

AB Natural killer activity of mouse spleen cells toward a human myeloid leukemia cell line, K562, can be enhanced by alloantisera directed against individual antigens in the H-2 region. By using a panel of 13 antisera (8 directed against antigens in the K and D regions and 5 directed against antigens in the I region) and four strains of mice (C57BL/6J, CBA, DBA/2, and A/J) it was found that certain antisera would **stimulate** target cell lysis by spleen cells only if the antisera had specificity for antigens which were a part of the haplotype represented on the spleen NK effector cells. Anti Ia antisera could **stimulate** the anti K562 **NK activity** of nude mouse spleen cells which lack mature T cells. Depletion of B cells and macrophages from nude spleen cells, by passing through a nylon-wool column also did not abolish the effect of anti-Ia antiserum. It appears likely therefore that the anti-Ia **antibodies** exert this effect directly on NK cells and that Ia antigens may be expressed on NK cells. Since the antisera directed against different antigens in H-2 complex irrespective of subregion specificity (K, D, or I) **stimulated** the **NK activity** of mouse spleen cells, the phenomenon offered an interesting **method** for testing the presence of a given alloantigen on mouse spleen cells. Log-dose response curves for the augmentation of lysis induced by appropriate alloantisera were linear over a dilution range of 1:320 to 1:5120. By using the dose-response curves, potency ratios of two preparations of antisera (directed against antigen 33 of the K region) could be successfully determined. Besides the K562 cell line, many human lymphoblastoid cell lines could also be used as target cells in this assay system.

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=> s NK activity
L27      14161 NK ACTIVITY

=> s l27 and increas?
L28       5812 L27 AND INCREAS?

=> s l28 and antibod?
L29       1482 L28 AND ANTIBOD?

=> s l29 and NK receptor
L30          9 L29 AND NK RECEPTOR

=> dup remove l30
PROCESSING COMPLETED FOR L30
L31           3 DUP REMOVE L30 (6 DUPLICATES REMOVED)

=> d l31 1-3 cbib abs

L31  ANSWER 1 OF 3  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
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2002:385219 Document No.: PREV200200385219. Normalization of natural killer cell function and phenotype with effective anti-HIV therapy and the role of IL-10. Parato, Karl G.; Kumar, Ashok; Badley, Andrew D.; Sanchez-Dardon, Jaime L.; Chambers, Kelley A.; Young, Charlene D.; Lim, Wilfred T.; Kravcik, Stephen; Cameron, D. William; Angel, Jonathan B. [Reprint author]. Division of Infectious Diseases, Ottawa Hospital, 501 Smyth Road, General Campus, Room G12, Ottawa, Ontario, K1H 8L6, Canada. jangel@ottawahospital.on.ca. AIDS (Hagerstown), (14 June, 2002) Vol. 16, No. 9, pp. 1251-1256. print.
CODEN: AIDSET. ISSN: 0269-9370. Language: English.

AB Objectives: Natural killer (NK) cell function is likely to be important in controlling HIV infection and opportunistic pathogens. We therefore evaluated NK function and phenotype over the course of antiretroviral therapy (ART) and examined the potential mechanisms of altered **NK activity** in HIV infection. Methods: We measured NK cell percentage, NK cytolytic activity (both by flow cytometry) and plasma IL-10 concentrations (by enzyme-linked immunosorbent assay) in 10 HIV-seropositive patients before and over one year of effective ART. To examine potential mechanisms of altered **NK activity**, we measured **NK receptor** expression in ART treated and untreated HIV-positive individuals by flow cytometry. As IL-10 enhances **NK activity**, we studied the effect of IL-10 on **NK receptor** expression and activity in peripheral blood mononuclear cells (PBMC) from HIV-seronegative individuals. Results: NK cytolytic activity was elevated in HIV infection and decreased with ART to levels observed in HIV-negative individuals. A greater proportion of NK cells from untreated HIV-positive individuals expressed the **NK receptors** CD158a and CD161 than either HIV-negative volunteers or effectively treated HIV-positive patients. NK cells from PBMC incubated with IL-10 demonstrated **increases** in CD158a, CD161 and CD94 expression and **increases** in cytolytic activity. The treatment-associated decrease in **NK activity** paralleled a decrease in IL-10 production. Conclusion: The observation that IL-10 alters **NK receptor** expression similar to that observed in HIV infection, and the fact that **NK receptor** expression and activity normalize in parallel with ART-induced reduction of circulating IL-10 levels supports a role for IL-10 in NK cell activity and HIV immunopathogenesis.

L31 ANSWER 2 OF 3 MEDLINE on STN DUPLICATE 1

2001454056. PubMed ID: 11499809. Renal carcinoma cell lines inhibit natural killer activity via the CD94 receptor molecule. Stanley A J; Gough M J; Banks R E; Selby P J; Patel P M. (ICRF Cancer Medicine Research Unit, St James's University Hospital, Leeds, UK.) Cancer immunology, immunotherapy : CII, (2001 Jul) 50 (5) 260-8. Journal code: 8605732. ISSN: 0340-7004. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB MHC class I molecules protect normal and transformed cells from lysis by natural killer (NK) cells through recognition of receptors expressed on leucocytes. Defects in NK cell activity and lymphokine activated killer (LAK) cell generation have been previously demonstrated in patients with renal cell carcinoma (RCC). However, to date, the importance of **NK receptor**/MHC class I interactions for immune evasion by RCC cells has not been described. In this study, human RCC cell lines (HTB46, HTB47, ACHN, CRL 1933 and HTB44) were found to be susceptible to lysis by both NK cells and interleukin-15 (IL-15)-derived LAK cells from normal donors in vitro. However, when NK cells were co-cultured with RCC cells their expression of the CD94 **NK receptor** molecule was significantly **increased** and their cytolytic activity against RCC targets was reduced. The cytolytic activity of NK cells was restored by the addition of IL-15, which further augmented the expression of CD94 on CD56+ NK cells. Disruption of **NK**

receptor-MHC class I interactions by the addition of blocking **antibodies** to CD94 had no effect on the lysis of K562 or HTB47 targets by NK cells. However, the sensitivity of HTB46 cells to NK-mediated lysis was **increased** by blocking the CD94 receptor molecule, but only when the NK cells had not been previously co-cultured with RCC cells. This was independent of the presence of IL-15. These results show that RCC cells can inhibit **NK activity** via CD94 and suggest that disruption of interactions between receptor and ligand on RCC cells in vivo may augment the immune response against tumours by innate effector cells.

L31 ANSWER 3 OF 3 MEDLINE on STN DUPLICATE 2
83259948. PubMed ID: 6872324. **Antibody**- and interferon-dependent killer cells are part of the NK cell receptor positive subpopulation of human peripheral blood cells. Ullberg M; Jondal M. Clinical and experimental immunology, (1983 Jul) 53 (1) 101-8. Journal code: 0057202. ISSN: 0009-9104. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Cytotoxicity by human non-adherent peripheral blood lymphocytes was analysed after effector cell activation with either interferon (IF) or by target cell specific IgG **antibodies** (T-IgG). Four different cell lines were used as target cells that differed in susceptibility to natural killer cell (**NK**) **activity**; a highly susceptible T cell line (Molt-4), a medium susceptible B lymphoma line (Daudi), a resistant B cell line established by Epstein-Barr virus transformation (LCL-LS) and a resistant mouse mastocytoma line (P815). Three different parameters influencing killing were investigated; lytic potential, target cell binding and efficiency of the lytic phase from which the absolute number of effector cells and their recycling capacity could be estimated. It was found that, when using human target cell lines, IF and T-IgG influenced the system in a similar way by activating the lytic phase and the effector cell recycling but not the early binding phase. With the NK resistant mouse mastocytoma cell line P815 a comparatively small target binding population was found which, however, **increased** markedly with T-IgG treatment. Taken together, the results indicate that the effector population responsible for **antibody**-induced killing belong to a subpopulation of cells that have the ability to spontaneously conjugate to the present target cells by virtue of naturally occuring undefined cell surface receptors designated NKR (**NK receptor**) and that the role of T-IgG in the present system is similar to that of IF. In contrast, if target cells are used that do not express binding structures for NKR receptors, T-IgG may also fulfill a receptor function through Fc receptors for IgG.

=> s NK receptor
L32 2053 NK RECEPTOR

=> s l32 and p30
L33 1 L32 AND P30

=> d l33 cbib abs

L33 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2003:357967 Document No.: PREV200300357967. Selective Expansion of **NK Receptor**-Positive Anti-Leukemia Cytotoxic T Cells from Leukemia Patients Treated with Hematopoietic Stem Cell Transplantation. Ogasawara, Masahiro [Reprint Author]; Ogawa, Takafumi [Reprint Author]; Imai, Kiyotoshi [Reprint Author]; Kobayashi, Naoki [Reprint Author]; Kiyama, Yoshio [Reprint Author]; Higa, Toshio [Reprint Author]; Tanaka, Junji [Reprint Author]; Imamura, Masahiro [Reprint Author]; Kasai, Masaharu [Reprint Author]. Department of Internal Medicine, Sapporo Hokuyu Hospital, Sapporo, Hokkaido, Japan. Blood, (November 16 2002) Vol. 100,

No. 11, pp. Abstract No. 3696. print.
Meeting Info.: 44th Annual Meeting of the American Society of Hematology. Philadelphia, PA, USA. December 06-10, 2002. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB The strategy to enhance the acquired and the innate immunity is a potent and an ideal immunotherapy for malignancies. Human invariant Valpha24+ natural killer T (NKT) cells have been found to play important roles on innate immunity by regulating various immune responses. These cells can be activated by glycolipids in a CD1d-dependent fashion. However, it is hard to expand NKT cells in vitro for a clinical use. As an alternative source, we are interested in CD3+ CD56+ cells which are thought to be a human counterpart of mouse type III NKT cells. In the present study, we investigated the characteristics, cytotoxic activity, expandability of CD3+ CD56+ cells from peripheral blood (PB) of leukemia patients who had been treated with hematopoietic stem cell transplantation (SCT). PB was collected from 11 patients with acute myelogenous leukemia (3), acute lymphocytic leukemia (3), chronic myelogenous leukemia (4) and myelodysplastic syndrome (1). All the patients were treated with allogeneic SCT (5 peripheral blood, 6 bone marrow). 5 healthy volunteers were involved as a control. Peripheral blood mononuclear cells (PBMCs), pre-incubated with IFNgamma (1000U/ml) for 1 day, were cultured with anti-CD3 antibody (50ng/ml) and IL2 (400U/ml) and continued to culture for 3 weeks by changing media every 3 to 4 days. FACS analysis showed that CD8+ and TCRalphabeta+ phenotypes were predominant among the cultured CD3+ CD56+ cells . Although these cells did not express CD16, CD57 and TCR Valpha24, approximately 30% cells expressed CD161, indicating that these cells shared the features of type III NKT cells. Morphologically, CD3+ CD56+ cells resembled large granular lymphocytes. Before culturing, the median proportions of CD3+ CD56+ cells in PBMCs were 5% (1-8%) in healthy volunteers and 3% (2-17%) in leukemia patients. A marked increase in the proportions of CD3+ CD56+ cells was observed after 21-day culture in both groups. The median proportions of CD3+ CD56+ cells increased to 62% (56-78%) in healthy volunteers and 58% (51-85%) in patients (not a significant difference). The proportions of CD3+ CD56+ cells were not significantly different in terms of the period following SCT or the source of stem cells. However, the proliferation of CD3+ CD56+ cells from patients was slightly decreased. The cytotoxic activity of CD3+ CD56+ cells was evaluated by a LDH release assay. CD3+ CD56+ cells from both healthy volunteers and patients lysed various leukemia cell lines including MOLT4, **P30**/OHK, HL60, Daudi and HLA-negative NK target K562. These cells lysed autologous and allogeneic leukemia cells but did not lyse autologous and allogeneic PHA-blasts, indicating that these cells have a potent HLA unrestricted cytotoxicity against leukemia cells. In conclusion, these results demonstrated that CD3+ CD56+ type III NKT-like cells, with a potent cytotoxicity against autologous and allogeneic leukemia cells, can be generated from leukemia patients treated with SCT. Utilization of these cells may be a useful therapeutic modality for leukemia patients especially to promote a graft-versus-leukemia effect of a donor lymphocyte infusion for the treatment of a relapse following SCT.

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=> s NKp30
L34         167 NKP30

=> s l34 and antibod?
L35          54 L34 AND ANTIBOD?

=> s l35 and NK activity
L36           0 L35 AND NK ACTIVITY

=> dup remove l35
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PROCESSING COMPLETED FOR L35
L37 20 DUP REMOVE L35 (34 DUPLICATES REMOVED)

=> d l37 1-20 cbib abs

L37 ANSWER 1 OF 20 MEDLINE on STN DUPLICATE 1
2004025706. PubMed ID: 14724446. Identification, cloning, and characterization of a novel rat natural killer receptor, RNKP30: a molecule expressed in liver allografts. Hsieh Christine L; Ogura Yasuhiro; Obara Hideaki; Ali Unzila A; Rodriguez Guadalupe M; Nepomuceno Ronald R; Martinez Olivia M; Krams Sheri M. (Department of Surgery and Program in Immunology, Stanford University School of Medicine, Stanford, California 94305-5492, USA.) Transplantation, (2004 Jan 15) 77 (1) 121-8. Journal code: 0132144. ISSN: 0041-1337. Pub. country: United States. Language: English.

AB BACKGROUND: As a component of the innate immune system, natural killer (NK) cells may play a significant role in the early events after solid-organ transplantation. Activated NK cells have been shown to infiltrate allografts in transplant models. To better understand NK cells and the role of NK cell receptors in transplantation, we have cloned and begun characterizing a novel rat molecule, rNKp30. METHODS: RNKp30 cDNA was cloned by 5' rapid amplification of cDNA ends polymerase chain reaction (PCR) and reverse transcriptase (RT)-PCR from mononuclear cells infiltrating a rejecting liver allograft. Southern blot analysis was used to determine the rNKp30 gene copy number. RT-PCR and Northern blotting were used to examine rNKp30 RNA expression in NK cells, multiple tissues, and liver grafts. Immunocytochemistry, immunoprecipitation, and Western blot analysis with two anti-rNKp30 polyclonal **antibodies**, CA680 and CA1071, were performed. Tunicamycin and endoglycosidase treatments determined the extent of rNKp30 glycosylation. RESULTS: RNKp30 is homologous to human and macaque **NKp30**. It is a single copy gene with five identified single-nucleotide polymorphisms. RNKp30 is expressed by NK cells and is detectable as a single transcript by Northern blot in normal spleen, lymph node, and lung tissues. RNKp30 is a variably N-glycosylated cell surface molecule with a protein backbone of approximately 21 kDa. Elevated transcript expression of rNKp30 is detected in both rejected and spontaneously accepted liver allografts, but not in syngeneic or cyclosporine A-treated allografts. CONCLUSIONS: RNKp30 is a glycosylated surface NK cell receptor with limited polymorphism. This putative activation receptor is expressed in liver allografts and may participate in the innate immune response after transplantation.

L37 ANSWER 2 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN
2003:117977 Document No. 138:168793 Plasmacytoid dendritic cell gene expression patterns and database for diagnostic and therapeutic uses. Lipford, Grayson B.; Wagner, Hermann; Zenke, Martin (Coley Pharmaceutical G.m.b.H., Germany; Max-Delbrueck-Centrum Fuer Molekulare Medizin). PCT Int. Appl. WO 2003012061 A2 20030213, 86 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US24410 20020801. PRIORITY: US 2001-PV309260 20010801.

AB The invention provides methods and compns. relating to a dendritic cell expression database. The database comprises expression patterns for a plurality of genes of both known and unknown function, in a purified population of plasmacytoid dendritic cells at both rest and activated

state. The methods of the invention comprise determination of marker mRNA expression by Northern anal., RT-PCR, or gene chip; or marker protein expression by FACS anal. to determine state of dendritic cells and to diagnose e.g. microbial infection or autoimmune diseases.

L37 ANSWER 3 OF 20 MEDLINE on STN DUPLICATE 2

2003553939. PubMed ID: 14635045. CD59 is physically and functionally associated with natural cytotoxicity receptors and activates human NK cell-mediated cytotoxicity. Marcenaro Emanuela; Augugliaro Raffaella; Falco Michela; Castriconi Roberta; Parolini Silvia; Sivori Simona; Romeo Elisa; Millo Romano; Moretta Lorenzo; Bottino Cristina; Moretta Alessandro. (Dipartimento di Medicina Sperimentale, Universita degli Studi di Genova, Genova, Italy.) European journal of immunology, (2003 Dec) 33 (12) 3367-76. Journal code: 1273201. ISSN: 0014-2980. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB Triggering of cytotoxicity in human NK cells is induced by the combined engagement of several triggering receptors. These include primary receptors such as NKG2D and the natural cytotoxicity receptors (NCR) **NKp30**, NKp46 and NKp44, while other molecules, including 2B4, NTB-A and NKp80, function as co-receptors. As reported in the present study, during an attempt to identify novel NK receptors or co-receptors, we found that CD59 functions as a co-receptor in human NK cell activation; engagement of CD59 by specific mAb delivers triggering signals to human NK cells, resulting in enhancement of cytotoxicity. Similar to other NK co-receptors, the triggering function of CD59, a glycosylphosphatidylinositol (GPI)-linked protein, depends on the simultaneous engagement of primary receptors such as NCR. Accordingly, CD59-dependent triggering was virtually restricted to NK cells expressing high surface densities of NKp46, and mAb-mediated modulation of NKp46 resulted in markedly decreased responses to anti-CD59 mAb. Biochemical analysis revealed that CD59 is physically associated with NKp46 and **NKp30**. Moreover, engagement of CD59 resulted in tyrosine phosphorylation of CD3zeta chains associated with these NCR, but not those associated with CD16. Thus, CD59-mediated costimulation of NK cells requires direct physical interaction of this GPI-linked protein with primary triggering NK receptors.

L37 ANSWER 4 OF 20 MEDLINE on STN DUPLICATE 3

2003393331. PubMed ID: 12750175. Expression and function of KIR and natural cytotoxicity receptors in NK-type lymphoproliferative diseases of granular lymphocytes. Zambello Renato; Falco Michela; Della Chiesa Mariella; Trentin Livio; Carollo Davide; Castriconi Roberta; Cannas Giovanna; Carlomagno Simona; Cabrelle Anna; Lamy Thierry; Agostini Carlo; Moretta Alessandro; Semenzato Gianpietro; Vitale Massimo. (Dipartimento di Medicina Sperimentale, Immunologia Clinica, Universita di Padova e Centro di Eccellenza per la Ricerca Biomedica Padova, Padua, Italy.) Blood, (2003 Sep 1) 102 (5) 1797-805. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Using monoclonal **antibodies** (mAbs) specific for different natural killer (NK) receptors, we studied the lymphocyte population from 18 patients with NK-type lymphoproliferative disease of granular lymphocytes (LDGL). The analysis of both resting and cultured NK cell populations demonstrated that these patients are frequently characterized by NK cells displaying a homogeneous staining with given anti-killer Ig-like receptor (anti-KIR) mAb (11 of 18 patients). In most patients NK cells were characterized by the CD94/NKG2A+ phenotype, whereas only a minor fraction of the cases expressed CD94/NKG2C. In 7 of these patients we could also assess the function of the various NK receptors. Remarkably those KIR molecules that, in each patient, homogeneously marked the NK cell expansion were found to display an activating function as determined by cross-linking with specific anti-KIR mAb. The KIR genotype analysis performed in 13 of 18 cases revealed that in NK-type LDGL certain

activating KIRs, as well as certain infrequent KIR genotypes, were detected with higher frequencies as compared to previously analyzed healthy donors. Moreover, most KIR genotypes included multiple genes coding for activating KIRs. The analysis of non-HLA-specific triggering receptors indicated that the natural cytotoxicity receptors (NKp46, **NKp30**) were expressed at significantly low levels in freshly drawn NK cells from most patients analyzed. However, in most instances the expression of NKp46 and **NKp30** could be up-regulated on culture in interleukin 2. Our data indicate that in NK-LDGL the expanded subset is frequently characterized by the expression of a given activating KIR, suggesting a direct role for these molecules in the pathogenetic mechanisms of this disorder.

L37 ANSWER 5 OF 20 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2003210499 EMBASE Selective cross-talk among natural cytotoxicity receptors in human natural killer cells. Augugliaro R.; Parolini S.; Castriconi R.; Marcenaro E.; Cantoni C.; Nanni M.; Moretta L.; Moretta A.; Bottino C.. A. Moretta, Dipartimento di Med. Sperimentale, Sezione di Istologia, Universita degli Studi di Genova, Via G.B. Marsano 10, I-16132 Genova, Italy. alemoret@unige.it. European Journal of Immunology 33/5 (1235-1241) 1 May 2003.
Refs: 31.
ISSN: 0014-2980. CODEN: EJIMAF. Pub. Country: Germany. Language: English. Summary Language: English.

AB The cytolytic activity of human natural killer cells is induced by several triggering cell surface receptors upon interaction with specific cellular ligands. These receptors include NKp46, **NKp30** and NKp44, collectively termed natural cytotoxicity receptors (NCR). Co-operation among NCR has been shown to occur for optimal recognition and killing of most tumor target get cells. In this study, we show that the mAb-mediated engagement and clustering of one or another NCR results in the activation of an identical set of tyrosine kinases. These kinases are included in the signaling cascade leading to tyrosine phosphorylation of different receptor-associated signal transducing molecules i.e. CD3ζ (associated with NKp46 and **NKp30**) and KARAP/DAP12 (associated with NKp44). In line with the notion that the engagement of inhibitory receptors prevents NCR-mediated responses, we show that the engagement of CD94/NKG2A virtually abrogates the tyrosine phosphorylation of the NCR-associated signaling molecules, i.e. it acts at the very early steps of the signaling cascade. Importantly, the engagement of a single NCR resulted in the activation of the signaling cascades associated with the other NCR. This "cross-talk" is confined to NKp46, **NKp30** and NKp44 since neither CD16- nor KIR2DS4-associated signaling polypeptides were phosphorylated following the NCR engagement. These results suggest that a functional cross-talk specifically occurs among different NCR, possibly resulting in the amplification of the activating signals.

L37 ANSWER 6 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN

2002:716321 Document No. 137:246527 Multivalent MHC constructs: Immunoanalysis, diagnosis and therapy. Winther, Lars; Petersen, Lars Oestergaard; Buus, Soeren; Schoeller, Joergen; Ruub, Erik; Aamellem, Oeystein (Dako A/S, Den.; Dynal Biotech Asa). PCT Int. Appl. WO 2002072631 A2 20020919, 304 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-DK169

20020313. PRIORITY: DK 2001-435 20010314; DK 2001-436 20010314; DK 2001-441 20010314; US 2001-PV275470 20010314; US 2001-PV275448 20010314; US 2001-PV275447 20010314.

AB The authors disclose MHC mol. constructs (classical and non-classical) conjugated to soluble or insol. carriers wherein the affinity and avidity of the constructs exceed that of comparable MHC tetramers. In one example, the construct is comprised of biotinylated HLA-A2 bound to FITC-labeled streptavidin conjugated to soluble derivatized dextran. The above construct loaded with MART-1 or influenza virus peptides was shown to effect T-cell activation at a lower concentration than. Also comprised by the present invention is the sample-mounted use of MHC mols., MHC mol. multimers, and MHC mol. constructs.

L37 ANSWER 7 OF 20 MEDLINE on STN DUPLICATE 4

2002654032. PubMed ID: 12414645. Major histocompatibility complex class I-related chain A and UL16-binding protein expression on tumor cell lines of different histotypes: analysis of tumor susceptibility to NKG2D-dependent natural killer cell cytotoxicity. Pende Daniela; Rivera Paola; Marcenaro Stefania; Chang Chien-Chung; Biassoni Roberto; Conte Romana; Kubin Marek; Cosman David; Ferrone Soldano; Moretta Lorenzo; Moretta Alessandro. (Istituto Nazionale per la Ricerca sul Cancro, 16132 Genova, Italy.) Cancer research, (2002 Nov 1) 62 (21) 6178-86. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB NKG2D, together with NKp46 and **NKp30**, represents a major triggering receptor involved in the induction of cytotoxicity by both resting and activated human natural killer cells. In this study, we analyzed the expression and the functional relevance of MHC class I-related chain A (MICA) and UL16 binding protein (ULBP), the major cellular ligands for human NKG2D, in human tumor cell lines of different histological origin. We show that MICA and ULBP are frequently coexpressed by carcinoma cell lines, whereas MICA is expressed more frequently than ULBP by melanoma cell lines. Interestingly, the MICA(-) ULBP(+) phenotype was detected in most T cell leukemia cell lines, whereas the MICA(-) ULBP(-) phenotype characterized all acute myeloid leukemia and most B-cell lymphoma cell lines analyzed. These results, together with functional experiments, based on monoclonal **antibody**-mediated blocking of either NKG2D or its ligands, showed that killing of certain MICA(-) cell tumors is at least in part NKG2D dependent. Indeed, leukemic T cells as well as certain B-cell lymphomas were killed in a NKG2D-dependent fashion upon recognition of ULBP molecules. Moreover, ULBP could induce NKG2D-mediated NK cell triggering also in tumors coexpressing MICA. Our data suggest that the involvement of NKG2D in natural killer cell-mediated cytotoxicity strictly correlates with the expression and the surface density of MICA and ULBP on target cell tumors of different histotypes.

L37 ANSWER 8 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN

2002:385429 Document No. 137:107211 Defective expression and function of natural killer cell-triggering receptors in patients with acute myeloid leukemia. Costello, Regis T.; Sivori, Simona; Marcenaro, Emanuela; Lafage-Pochitaloff, Marina; Mozziconacci, Marie-Joelle; Reviron, Denis; Gastaut, Jean-Albert; Pende, Daniela; Olive, Daniel; Moretta, Alessandro (Unite d'Immunologie des Tumeurs Departement d'Hematologie, Institut Paoli-Calmettes, Universite de la Mediterranee, Marseille, Fr.). Blood, 99(10), 3661-3667 (English) 2002. CODEN: BLOOAW. ISSN: 0006-4971. Publisher: American Society of Hematology.

AB The cytolytic function of natural killer (NK) cells is induced by the engagement of a series of activating receptors and coreceptors some of which have recently been identified and collectively termed natural cytotoxicity receptors (NCRs). Here, the authors analyzed the cytolytic function of NK cells obtained from patients with acute myeloid leukemia

(AML). In sharp contrast with healthy donors, in most (16 of 18) patients with AML the majority of NK cells displayed low NCR surface d. (NCRdull). This phenotype correlated with a weak cytolytic activity against autologous leukemic cells that could not be reversed by the monoclonal **antibody**-mediated disruption of HLA class I/killer Ig-like receptor interaction. The remaining 2 patients were characterized by NK cells having an NCRbright phenotype. Surprisingly, although displaying NCR-mediated cytolytic activity, these NCRbright NK cells were unable to kill autologous leukemic blasts. Importantly, the leukemic blasts from these 2 patients were also resistant to lysis mediated by normal NCRbright allogeneic NK cells. The authors' study suggests that in most instances the inability of NK cells to kill autologous leukemic blasts is consequent to low NCR surface expression. In few cases, however, this failure appears to involve a mechanism of tumor escape based on down-regulation of ligands relevant for NCR-mediated target cell recognition.

L37 ANSWER 9 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN
2002:198984 Document No. 136:339333 Analysis of natural killer cells in TAP2-deficient patients: expression of functional triggering receptors and evidence for the existence of inhibitory receptor(s) that prevent lysis of normal autologous cells. Vitale, Massimo; Zimmer, Jacques; Castriconi, Roberta; Hanau, Daniel; Donato, Lionel; Bottino, Cristina; Moretta, Lorenzo; De la Salle, Henri; Moretta, Alessandro (Instituto Nazionale per la Ricerca sul Cancro, Genoa, Italy). Blood, 99(5), 1723-1729 (English) 2002. CODEN: BLOOAW. ISSN: 0006-4971. Publisher: American Society of Hematology.

AB Natural killer (NK) cells are characterized by the ability to kill cells that lack HLA class I mols. while sparing autologous normal (HLA class I+) cells. However, patients with transporter-associated antigen processing (TAP) deficiency, though displaying strong redns. of HLA class I surface expression, in most instances do not experience NK-mediated autoimmune phenomena. A possible mechanism by which TAP-/- NK cells avoid autoreactivity against autologous HLA class I-deficient cells could be based on either quant. or qual. defects of surface receptors involved in NK cell triggering. In this study the authors show that NK cells derived from 2 patients with TAP2-/- express normal levels of all known triggering receptors. As revealed by the anal. of polyclonal and clonal NK cells, these receptors display normal functional capabilities and allow the killing of a panel of NK-susceptible targets, including autologous B-LCLs. On the other hand, TAP2-/- NK cells were unable to kill either allogeneic (HLA class I+) or autologous (HLA class I-) phytohemagglutinin (PHA) blasts even in the presence of anti-HLA class I monoclonal **antibody**. These data suggest that TAP2-/- NK cells express still unknown inhibitory receptor(s) capable of down-regulating the NK cell cytotoxicity on binding to surface ligand(s) expressed by T cell blasts. Functional analyses, both at the polyclonal and at the clonal level, are consistent with the concept that the putative inhibitory receptor is expressed by virtually all TAP2-/- NK cells, whereas it is present only in rare NK cells from healthy persons. Another possibility would be that TAP2-/- NK cells are missing a still unidentified triggering receptor involved in NK cell-mediated killing of PHA blasts.

L37 ANSWER 10 OF 20 MEDLINE on STN DUPLICATE 5
2002157915. PubMed ID: 11828009. Human dendritic cells activate resting natural killer (NK) cells and are recognized via the **NKp30** receptor by activated NK cells. Ferlazzo Guido; Tsang Ming L; Moretta Lorenzo; Melioli Giovanni; Steinman Ralph M; Munz Christian. (Laboratorio di Immunoterapia Cellulare, Unita di Immunologia, Istituto Nazionale per la Ricerca sul Cancro, 16132 Genova, Italy.. ferlazzo@cba.unige.it) . Journal of experimental medicine, (2002 Feb 4) 195 (3) 343-51. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB During the innate response to many inflammatory and infectious stimuli, dendritic cells (DCs) undergo a differentiation process termed maturation. Mature DCs activate antigen-specific naive T cells. Here we show that both immature and mature DCs activate resting human natural killer (NK) cells. Within 1 wk the NK cells increase two-- to fourfold in numbers, start secreting interferon (IFN)-gamma, and acquire cytolytic activity against the classical NK target LCL721.221. The DC-activated NK cells then kill immature DCs efficiently, even though the latter express substantial levels of major histocompatibility complex (MHC) class I. Similar results are seen with interleukin (IL)-2--activated NK cell lines and clones, i.e., these NK cells kill and secrete IFN-gamma in response to immature DCs. Mature DCs are protected from activated NK lysis, but lysis takes place if the NK inhibitory signal is blocked by a human histocompatibility leukocyte antigen (HLA)-A,B,C--specific **antibody**. The NK activating signal mainly involves the **NKp30** natural cytotoxicity receptor, and not the NKp46 or NKp44 receptor. However, both immature and mature DCs seem to use a **NKp30** independent mechanism to act as potent stimulators for resting NK cells. We suggest that DCs are able to control directly the expansion of NK cells and that the lysis of immature DCs can regulate the afferent limb of innate and adaptive immunity.

L37 ANSWER 11 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN

2001:380770 Document No. 135:4465 cDNA encoding novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells, and **antibodies** that identify the same. Moretta, Alessandro; Bottino, Cristina; Biassoni, Roberto (Innate Pharma S.A.S., Fr.; Universita Di Genova). PCT Int. Appl. WO 2001036630 A2 20010525, 83 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-EP11697 20001115. PRIORITY: US 1999-440514 19991115; CA 1999-2288307 19991115.

AB The invention relates to a cDNA sequence encoding a novel receptor termed **NKp30** of human. The receptor selectively expressed by all mature NK cells and that is involved in human natural cytotoxicity as an activatory receptor, to new **antibodies** that bind to the **NKp30** structure, and to the pharmaceutical and medicinal uses thereof.

L37 ANSWER 12 OF 20 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2002012827 EMBASE Identification, molecular cloning and functional characterization of NKp46 and **NKp30** natural cytotoxicity receptors in Macaca fascicularis NK cells. De Maria A.; Biassoni R.; Fogli M.; Rizzi M.; Cantoni C.; Costa P.; Conte R.; Mavilio D.; Ensoli B.; Cafaro A.; Moretta A.; Moretta L.. A. De Maria, Dipartimento di Medicina Interna, Immunology of Infectious Dis. Unit, University of Genova, 10 Largo R. Benzi, I-Genova 16132, Italy. de-maria@unige.it. European Journal of Immunology 31/12 (3546-3556) 2001.
Refs: 36.
ISSN: 0014-2980. CODEN: EJIMAF. Pub. Country: Germany. Language: English. Summary Language: English.

AB Natural killer (NK) cell recognition and function in humans is regulated by multiple cell surface receptors. The "on" signal leading to NK cell triggering is primarily mediated by natural cytotoxicity receptors (NCR). Analysis of NK cells in primate animal models is of particular relevance because NK cells may play an essential role in host defenses against

infections. We analyzed Macaca fascicularis PBMC and in vitro-derived NK cell populations and clones by cytofluorometry, using a wide panel of mAb, and by cytolytic activity assays. In addition, RT-PCR strategy and transient transfections were used to isolate M. fascicularis NCR. NCR-specific mAb reactivity (anti-NKp46 and anti-**NKp30**) was present on M. fascicularis PBMC and on NK cell cultures. Macaque NCR were functional in both redirected killing and in mAb-mediated masking assays. Cloning of macNKp46 and macNKp30 NCR homologous genes showed a high sequence similarity (86% and 88%, respectively) with their human counterparts. Attempts at identifying NKp44 surface reactivity and at cloning the macaque homologue were unsuccessful. NKp46 and **NKp30** NCRs, but not NKp44, are highly conserved in M. fascicularis NK cells. This suggests the possibility of a staged appearance of the NCR during phylogenesis and provides a useful tool for the study of natural immunity correlates of protection in primate SIV/SHIV infection models.

L37 ANSWER 13 OF 20 MEDLINE on STN

2001493031. PubMed ID: 11536166. Recognition of viral hemagglutinins by NKp44 but not by **NKp30**. Arnon T I; Lev M; Katz G; Chernobrov Y; Porgador A; Mandelboim O. (The Lautenberg Center for General and Tumor Immunology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.) European journal of immunology, (2001 Sep) 31 (9) 2680-9. Journal code: 1273201. ISSN: 0014-2980. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB Natural killer (NK) cells destroy virus-infected and tumor cells without prior antigen stimulation. The NK cell cytotoxicity is regulated in large part by the expression of NK cell receptors that are able to bind major histocompatibility complex (MHC) class I glycoproteins. NK cells also express lysis triggering receptors specific for non-MHC ligands, including **NKp30**, NKp44, NKp46 and CD16. However, the nature of their ligands, recognized on target cells, is undefined. We have recently shown that the NKp46 protein, but not the CD16 protein, recognizes the hemagglutinin (HA) of influenza virus (IV) and the hemagglutinin-neuraminidase (HN) of Sendai virus (SV), and that the recognition of HA from IV requires the sialylation of NKp46 oligosaccharides. We have also demonstrated that binding of NKp46 to HA of IV is required for lysis of cells expressing the corresponding glycoproteins by a substantial subset of NK clones. Here we show that NKp44, but not **NKp30**, can also recognize the HA of both IV and SV and that the recognition of IV HA requires the sialylation of the NKp44 receptor in a similar way to that of NKp46. SV infection of 721.221 cells expressing MHC class I proteinsresulted in the abrogation of the inhibition by NK clones expressing high levels of NKp44. In addition, the binding of NKp44 to HA improves the ability of some NK clones to lyse IV infected cells.

L37 ANSWER 14 OF 20 MEDLINE on STN DUPLICATE 6

2001311751. PubMed ID: 11385609. NK cell-mediated lysis of autologous antigen-presenting cells is triggered by the engagement of the phosphatidylinositol 3-kinase upon ligation of the natural cytotoxicity receptors **NKp30** and NKp46. Spaggiari G M; Carosio R; Pende D; Marcenaro S; Rivera P; Zocchi M R; Moretta L; Poggi A. (Laboratory of Immunology, National Cancer Research Institute, Genova, Italy.) European journal of immunology, (2001 Jun) 31 (6) 1656-65. Journal code: 1273201. ISSN: 0014-2980. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB Interleukin-2 (IL-2)-activated polyclonal or clonal NK cells lysed autologous antigen presenting cells (APC) through the engagement of the natural cytotoxicity receptors (NCR) **NKp30** and NKp46. NK cell-mediated cytolysis of APC correlated with the surface density of these NCR. Indeed, NK cell clones bearing low amounts of **NKp30** and NKp46 did not lyse autologous APC, whereas NK cell clones with bright expression of these NCR efficiently killed autologous APC. Upon masking

of **NKp30** or NKp46 by specific monoclonal **antibodies** a strong reduction (by 50%) of APC lysis could be detected and the complete inhibition was achieved by the simultaneous masking of these NCR. Interestingly, NK cell-mediated APC lysis was impaired by the phosphatidylinositol 3-kinase (PI-3 K) inhibitors LY294002 or wortmannin. Similarly, these drugs strongly reduced NK cell activation triggered by **NKp30** or NKp46 in a re-directed killing assay as well as the activation of Akt/PKB, substrate of PI-3 K, induced by the engagement of these receptors. Altogether, these findings strongly suggest that NCR are responsible for the killing of autologous APC through the activation of PI-3 K.

L37 ANSWER 15 OF 20 MEDLINE on STN DUPLICATE 7

2001434402. PubMed ID: 11244035. Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. Moretta A; Bottino C; Vitale M; Pende D; Cantoni C; Mingari M C; Biassoni R; Moretta L. (Dipartimento di Medicina Sperimentale, Universita degli Studi di Genova, Italy.. alemoret@unige.it) . Annual review of immunology, (2001) 19 197-223. Ref: 127. Journal code: 8309206. ISSN: 0732-0582. Pub. country: United States. Language: English.

AB Natural killer cells can discriminate between normal cells and cells that do not express adequate amounts of major histocompatibility complex (MHC) class I molecules. The discovery, both in mouse and in human, of MHC-specific inhibitory receptors clarified the molecular basis of this important NK cell function. However, the triggering receptors responsible for positive NK cell stimulation remained elusive until recently. Some of these receptors have now been identified in humans, thus shedding some light on the molecular mechanisms involved in NK cell activation during the process of natural cytotoxicity. Three novel, NK-specific, triggering surface molecules (NKp46, **NKp30**, and NKp44) have been identified. They represent the first members of a novel emerging group of receptors collectively termed natural cytotoxicity receptors (NCR). Monoclonal **antibodies** (mAbs) to NCR block to differing extents the NK-mediated lysis of various tumors. Moreover, lysis of certain tumors can be virtually abrogated by the simultaneous masking of the three NCRs. There is a coordinated surface expression of the three NCRs, their surface density varying in different individuals and also in the NK cells isolated from a given individual. A direct correlation exists between the surface density of NCR and the ability of NK cells to kill various tumors. NKp46 is the only NCR involved in human NK-mediated killing of murine target cells. Accordingly, a homologue of NKp46 has been detected in mouse. Molecular cloning of NCR revealed novel members of the Ig superfamily displaying a low degree of similarity to each other and to known human molecules. NCRs are coupled to different signal transducing adaptor proteins, including CD3 zeta, Fc epsilon RI gamma, and KARAP/DAP12. Another triggering NK receptor is NKG2D. It appears to play either a complementary or a synergistic role with NCRs. Thus, the triggering of NK cells in the process of tumor cell lysis may often depend on the concerted action of NCR and NKG2D. In some instances, however, it may uniquely depend upon the activity of NCR or NKG2D only. Strict NKG2D-dependency can be appreciated using clones that, in spite of their NCR(dull) phenotype, efficiently lyse certain epithelial tumors or leukemic cell lines. Other triggering surface molecules including 2B4 and the novel NKp80 appear to function as coreceptors rather than as true receptors. Indeed, they can induce natural cytotoxicity only when co-engaged with a triggering receptor. While an altered expression or function of NCR or NKG2D is being explored as a possible cause of immunological disorders, 2B4 dysfunction has already been associated with a severe form of immunodeficiency. Indeed, in patients with the X-linked lymphoproliferative disease, the inability to control Epstein-Barr virus infections may be consequent to a major dysfunction of 2B4 that exerts inhibitory instead of activating functions.

L37 ANSWER 16 OF 20 MEDLINE on STN
2001106858. PubMed ID: 11137207. Triggering receptors involved in natural killer cell-mediated cytotoxicity against choriocarcinoma cell lines. Sivori S; Parolini S; Marcenaro E; Millo R; Bottino C; Moretta A. (Dipartimento di Medicina Sperimentale, Universita di Genova, Genova, Italy.) Human immunology, (2000 Nov) 61 (11) 1055-8. Journal code: 8010936. ISSN: 0198-8859. Pub. country: United States. Language: English.

AB The lack of classical HLA-class I molecules on trophoblast is necessary to prevent allorecognition by maternal CTL, but may induce activation of NK cells. A protective role against NK cells equipped of suitable inhibitory receptors has been proposed for nonclassical HLA-class I molecules including HLA-E and HLA-G. In the present study we show that the NK-mediated killing of two choriocarcinoma cell lines, JAR and JEG3, is induced upon engagement of natural cytotoxicity receptors (NCR) with their specific ligands. In particular, we show that NKp44, a triggering receptor expressed at the NK cell surface only after in vitro culture in the presence of IL-2, plays a central role in triggering NK cytotoxicity against trophoblast cells. Also NKp46 appear to contribute to this function by cooperating with NKp44. On the other hand, other triggering receptors such as **NKp30**, 2B4, and NKG2D are not involved in killing of choriocarcinoma. Our findings suggest that resistance of trophoblast to NK-mediated cytotoxicity is the result of insufficient activating interactions between the various triggering NK receptors and their target cell ligands. On the other hand, the interaction of nonclassical HLA class I molecules with inhibitory NK receptors appears to play only a marginal role in regulating the susceptibility of choriocarcinoma to NK mediated cytotoxicity.

L37 ANSWER 17 OF 20 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 8
2000144058 EMBASE 2B4 functions as a co-receptor in human NK cell activation. Sivori S.; Parolini S.; Falco M.; Marcenaro E.; Biassoni R.; Bottino C.; Moretta L.; Moretta A.. A. Moretta, Dipartimento Medicina Sperimentale, Sezione di Istologia, Via G.B. Marsano 10, I-16132 Genova, Italy. alemoret@unige.it. European Journal of Immunology 30/3 (787-793) 2000. Refs: 24.
ISSN: 0014-2980. CODEN: EJIMAF. Pub. Country: Germany. Language: English. Summary Language: English.

AB Natural cytotoxicity receptors (NKp46, NKp44 and **NKp30**) play a predominant role in human NK cell triggering during natural cytotoxicity. Human 2B4 also induced NK cell activation in redirected killing assays using anti-2B4 monoclonal **antibodies** (mAb) and murine targets. Since this effect was confined to a fraction of NK cells, this suggested a functional heterogeneity of 2B4 molecules. Here we show that activation via 2B4 in redirected killing against murine targets is strictly dependent upon the engagement of NKp46 by murine ligand(s) on target cells. Thus, NK cell clones expressing high surface density of NKp46 (NKp46(bright)) were triggered by anti-2B4 mAb, whereas NKp46(dull) clones were not although they expressed a comparable surface density of 2B4. mAb-mediated modulation of NKp46 molecules in NKp46(bright) clones had no effect on the expression of 2B4 while it rendered cells unresponsive to anti-2B4 mAb. Finally, anti-2B4 mAb could induce NK cell triggering in NKp46(dull) clones provided that suboptimal doses of anti-NKp44 or anti-CD16 mAb were added to the redirected killing assay. These results indicate that differences in responses do not reflect a functional heterogeneity of 2B4 but rather depend on the co-engagement of triggering receptors.

L37 ANSWER 18 OF 20 MEDLINE on STN DUPLICATE 9
2000429220. PubMed ID: 10934222. X-linked lymphoproliferative disease. 2B4 molecules displaying inhibitory rather than activating function are responsible for the inability of natural killer cells to kill Epstein-Barr

virus-infected cells. Parolini S; Bottino C; Falco M; Augugliaro R; Giliani S; Franceschini R; Ochs H D; Wolf H; Bonnefoy J Y; Biassoni R; Moretta L; Notarangelo L D; Moretta A. (Dipartimento di Scienze Biomediche e Biotecnologie, Universita di Brescia, 25123 Brescia, Italy.) Journal of experimental medicine, (2000 Aug 7) 192 (3) 337-46. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB 2B4 is a surface molecule involved in activation of the natural killer (NK) cell-mediated cytotoxicity. It binds a protein termed Src homology 2 domain-containing protein (SH2D1A) or signaling lymphocyte activation molecule (SLAM)-associated protein (SAP), which in turn has been proposed to function as a regulator of the 2B4-associated signal transduction pathway. In this study, we analyzed patients with X-linked lymphoproliferative disease (XLP), a severe inherited immunodeficiency characterized by critical mutations in the SH2D1A gene and by the inability to control Epstein-Barr virus (EBV) infections. We show that, in these patients, 2B4 not only fails to transduce triggering signals, but also mediates a sharp inhibition of the NK-mediated cytolysis. Other receptors involved in NK cell triggering, including CD16, NKp46, NKp44, and **NKp30**, displayed a normal functional capability. However, their activating function was inhibited upon engagement of 2B4 molecules. CD48, the natural ligand of 2B4, is highly expressed on the surface of EBV(+) B cell lines. Remarkably, NK cells from XLP patients could not kill EBV(+) B cell lines. This failure was found to be the consequence of inhibitory signals generated by the interaction between 2B4 and CD48, as the **antibody**-mediated disruption of the 2B4-CD48 interaction restored lysis of EBV(+) target cells lacking human histocompatibility leukocyte antigen (HLA) class I molecules. In the case of autologous or allogeneic (HLA class I(+)) EBV(+) lymphoblastoid cell lines, restoration of lysis was achieved only by the simultaneous disruption of 2B4-CD48 and NK receptor-HLA class I interactions. Molecular analysis revealed that 2B4 molecules isolated from either XLP or normal NK cells were identical. As expected, in XLP-NK cells, 2B4 did not associate with SH2D1A, whereas similar to 2B4 molecules isolated from normal NK cells, it did associate with Src homology 2 domain-containing phosphatase 1.

L37 ANSWER 19 OF 20 MEDLINE on STN

2000314631. PubMed ID: 10854660. Involvement of natural cytotoxicity receptors in human natural killer cell-mediated lysis of neuroblastoma and glioblastoma cell lines. Sivori S; Parolini S; Marcenaro E; Castriconi R; Pende D; Millo R; Moretta A. (Dipartimento di Medicina Sperimentale (DIMES), Sezione di Istologia, Universita di Genova, Via Marsano 10, 16132, Genova, Italy.) Journal of neuroimmunology, (2000 Jul 24) 107 (2) 220-5. Journal code: 8109498. ISSN: 0165-5728. Pub. country: Netherlands. Language: English.

AB The surface receptors involved in natural killer (NK) cell triggering during the process of target cell lysis have been at least in part identified. These are members of a novel family of receptors that has been termed natural cytotoxicity receptors (NCR). The first three members of this emerging group of receptors are the NKp46, NKp44 and **NKp30** molecules that all belong to the immunoglobulin superfamily. Blocking of these receptors inhibits NK-mediated cytotoxicity against a wide variety of tumor target cells. In the present study, we show that these NCR are also involved in NK-mediated killing of tumor cells of neural origin. Glioblastoma and neuroblastoma target cells were efficiently killed by all NK clones analyzed since little protection from NK lysis was mediated by HLA class I molecules. Blocking of one or another NCR inhibited cytotoxicity; however, optimal inhibition was only observed when the three receptors were blocked simultaneously. A sharp difference in cytotoxicity against neural tumors was demonstrated between NCR(bright) and NCR(dull) NK clones, further supporting the notion that NCR play a critical role in the induction of cytotoxicity against tumor target cells of different histotype. Finally, our data also indicate that CD16 does not function as

a triggering receptor involved in lysis of neural tumors since no difference in cytotoxicity could be substantiated between CD16(+) and CD16(-) NK clones and no correlation could be detected between the NCR(bright)/NCR(dull) phenotype and CD16 expression.

L37 ANSWER 20 OF 20 MEDLINE on STN DUPLICATE 10
2000029809. PubMed ID: 10562324. Identification and molecular characterization of **NKp30**, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. Pende D; Parolini S; Pessino A; Sivori S; Augugliaro R; Morelli L; Marcenaro E; Accame L; Malaspina A; Biassoni R; Bottino C; Moretta L; Moretta A. (Istituto Nazionale per la Ricerca sul Cancro, 16132 Genova, Italy.) Journal of experimental medicine, (1999 Nov 15) 190 (10) 1505-16. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB Two major receptors involved in human natural cytotoxicity, NKp46 and NKp44, have recently been identified. However, experimental evidence suggested the existence of additional such receptor(s). In this study, by the generation of monoclonal **antibodies** (mAbs), we identified **NKp30**, a novel 30-kD triggering receptor selectively expressed by all resting and activated human natural killer (NK) cells. Although mAb-mediated cross-linking of **NKp30** induces strong NK cell activation, mAb-mediated masking inhibits the NK cytotoxicity against normal or tumor target cells. **NKp30** cooperates with NKp46 and/or NKp44 in the induction of NK-mediated cytotoxicity against the majority of target cells, whereas it represents the major triggering receptor in the killing of certain tumors. This novel receptor is associated with CD3zeta chains that become tyrosine phosphorylated upon sodium pervanadate treatment of NK cells. Molecular cloning of **NKp30** cDNA revealed a member of the immunoglobulin superfamily, characterized by a single V-type domain and a charged residue in the transmembrane portion. Moreover, we show that **NKp30** is encoded by the previously identified 1C7 gene, for which the function and the cellular distribution of the putative product were not identified in previous studies.

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=> s (moretta a?/au or bottino c?/au or biassoni r?/au)
L38       1923 (MORETTA A?/AU OR BOTTINO C?/AU OR BIASSONI R?/AU)

=> s l38 and NK antibod?
L39          0 L38 AND NK ANTIBOD?

=> s 138 and NK receptor
L40        187 L38 AND NK RECEPTOR

=> s l140 and human NK cell
L140 NOT FOUND
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of L-numbers, enter DISPLAY HISTORY at an arrow prompt (=>).

=> s 140 and human NK cell
   3 FILES SEARCHED...
L41         38 L40 AND HUMAN NK CELL

=> dup remove 141
PROCESSING COMPLETED FOR L41
L42          16 DUP REMOVE L41 (22 DUPLICATES REMOVED)

=> d 142 1-16 cbib abs

L42 ANSWER 1 OF 16     MEDLINE on STN                  DUPLICATE 1
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2003553939. PubMed ID: 14635045. CD59 is physically and functionally associated with natural cytotoxicity receptors and activates **human NK cell**-mediated cytotoxicity. Marcenaro Emanuela; Augugliaro Raffaella; Falco Michela; Castriconi Roberta; Parolini Silvia; Sivori Simona; Romeo Elisa; Millo Romano; Moretta Lorenzo; **Bottino Cristina; Moretta Alessandro.** (Dipartimento di Medicina Sperimentale, Universita degli Studi di Genova, Genova, Italy.) European journal of immunology, (2003 Dec) 33 (12) 3367-76. Journal code: 1273201. ISSN: 0014-2980. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB Triggering of cytotoxicity in **human NK cells** is induced by the combined engagement of several triggering receptors. These include primary receptors such as NKG2D and the natural cytotoxicity receptors (NCR) NKp30, NKp46 and NKp44, while other molecules, including 2B4, NTB-A and NKp80, function as co-receptors. As reported in the present study, during an attempt to identify novel **NK receptors** or co-receptors, we found that CD59 functions as a co-receptor in **human NK cell** activation; engagement of CD59 by specific mAb delivers triggering signals to **human NK cells**, resulting in enhancement of cytotoxicity. Similar to other NK co-receptors, the triggering function of CD59, a glycosylphosphatidylinositol (GPI)-linked protein, depends on the simultaneous engagement of primary receptors such as NCR. Accordingly, CD59-dependent triggering was virtually restricted to NK cells expressing high surface densities of NKp46, and mAb-mediated modulation of NKp46 resulted in markedly decreased responses to anti-CD59 mAb. Biochemical analysis revealed that CD59 is physically associated with NKp46 and NKp30. Moreover, engagement of CD59 resulted in tyrosine phosphorylation of CD3zeta chains associated with these NCR, but not those associated with CD16. Thus, CD59-mediated costimulation of NK cells requires direct physical interaction of this GPI-linked protein with primary triggering **NK receptors**.

L42 ANSWER 2 OF 16 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 2

2003:569864 Document No.: PREV200300562841. Surface receptors and functional interactions of human natural killer cells: From bench to the clinic. Moretta, L. [Reprint Author]; **Bottino, C.**; Ferlazzo, G.; Pende, D.; Melioli, G.; Mingari, M. C.; **Moretta, A.**. Dipartimento di Medicina Sperimentale, Universita degli Studi di Genova, Via L. B. Alberti 2, 16132, Genova, Italy. lorenzomoretta@ospedale-gaslini.ge.it. CMLS Cellular and Molecular Life Sciences, (October 2003) Vol. 60, No. 10, pp. 2139-2146. print.
ISSN: 1420-682X. Language: English.

AB The past 10 years have witnessed dramatic progress in our understanding of how natural killer (NK) cells function and their role in innate immunity. Thanks to an array of inhibitory receptors specific for different HLA class I molecules, **human NK cells** can sense the decrease or loss of even single alleles at the cell surface. This represents a typical condition of a potential danger, i.e. the presence of tumor or virally infected cells. NK cell triggering and lysis of these cells is mediated by several activating receptors and coreceptors that have recently been identified and cloned. While normal cells are usually resistant to NK-mediated attack, a remarkable exception is represented by dendritic cells (DCs). In their immature form they are susceptible to NK-mediated lysis because of the expression of low levels of surface HLA class I molecules. The process of DC maturation (mDCs) is characterized by the surface expression of high levels of HLA class I molecules. Accordingly, mDCs become resistant to NK cells. A recent major breakthrough highlighted the role played by donor NK cells in allogenic bone marrow transplantation to cure acute myeloid leukemias. 'Allore-active' NK cells derived from donor hematopoietic precursors not

only prevented leukemic relapses, but also prevented graft rejection and graft-versus-host disease.

L42 ANSWER 3 OF 16 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2003:786000 The Genuine Article (R) Number: 718GJ. Natural killer cell-triggering receptors in patients with acute leukaemia. Fauriat C; Marcenaro E; Sivori S; Rey J; Gastaut J A; **Moretta A**; Olive D; Costello R T (Reprint). Inst J Paoli I Calmettes, 232 Bd St Marguerite, F-13009 Marseille, France (Reprint); Inst J Paoli I Calmettes, F-13009 Marseille, France; Fac Med Marseille, F-13385 Marseille, France; Univ Mediterranee, Marseille, France; Univ Genoa, Sez Istol, Dipartimento Med Sperimentale, Lab Immunol Mol, Genoa, Italy; Ist Giannina Gaslini, I-16148 Genoa, Italy. LEUKEMIA & LYMPHOMA (SEP 2003) Vol. 44, No. 10, pp. 1683-1689. Publisher: TAYLOR & FRANCIS LTD. 4 PARK SQUARE, MILTON PARK, ABINGDON OX14 4RN, OXON, ENGLAND. ISSN: 1042-8194. Pub. country: France; Italy. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Human natural killer (NK) cells are potent effectors involved in destruction of virus infected cells and tumours. Their cytolytic function is regulated by surface receptors that either inhibit or increase the NK-mediated cytotoxicity. Under physiological conditions, NK cells recognize major histocompatibility complex (MHC)-class I molecules through surface receptors delivering signals that inhibit NK cells function. Nonetheless, the "missing self hypothesis", i.e. the release of an inhibitory signal by the interaction between HLA I-specific inhibitory receptors and their ligands, is not sufficient to entirely explain the regulation of NK cytotoxicity. Activating and co-receptors also play a central role in NK cell activation. In the haematology field, several lines of evidence suggest that NKs participate to the anti-leukaemia immune response: (1) leukaemic cells have down-regulated HLA-class I molecule expression and putative allele loss, (2) several reports have indicated an inverse relationship between NK cell number or activity and prognosis in acute leukaemia, (3) NK-cell activity dependent immunodeficiency syndromes are associated with an increased frequency of lymphoid haematological malignancies, (4) recent data support a role for NK cells in the graft-versus-leukaemia effect observed in allogeneic bone marrow transplantation. All these data raise several questions. How NK cells kill leukaemic targets, and how can leukaemia escape from innate immunity surveillance? What are the therapeutic possibilities to manipulate **NK receptor**-ligand interaction in order to increase leukaemia cell destruction? The responses to these questions will contribute to immunotherapy advancements in leukaemia and more generally in cancer.

L42 ANSWER 4 OF 16 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2003:756422 The Genuine Article (R) Number: 716XK. Update on natural killer cells: Cross-talk with dendritic cells and role in the cure of acute myeloid leukemias. Moretta L (Reprint); Ferlazzo G; Mingari M C; Melioli G; **Moretta A**. Ist Giannina Gaslini, L Gerolamo Gaslini 5, I-16147 Genoa, Italy (Reprint); Ist Giannina Gaslini, I-16147 Genoa, Italy; Univ Genoa, Dipartimento Med Sperimentale, I-16132 Genoa, Italy; Univ Genoa, Ctr Eccellenza Ric Biomed, I-16132 Genoa, Italy; Ist Nazl Ric Canc, I-16132 Genoa, Italy; Univ Genoa, Dipartimento Oncol Biol & Genet, I-16132 Genoa, Italy. CANCER JOURNAL (JUL-AUG 2003) Vol. 9, No. 4, pp. 232-237. Publisher: JONES AND BARTLETT PUBLISHERS. 40 TALL PINE DR, SUDBURY, MA 01776 USA. ISSN: 1528-9117. Pub. country: Italy. Language: English.

L42 ANSWER 5 OF 16 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2003:691867 The Genuine Article (R) Number: 709NL. Cellular and molecular basis of natural killer and natural killer-like activity. Moretta L (Reprint); Mingari M C; **Bottino C**; Pende D; **Biassoni R**

; **Moretta A.** Ist Giannina Gaslini, Direttore Sci, L Go Gerolamo Gaslini 5, I-16147 Genoa, Italy (Reprint); Univ Genoa, Dipartimento Med Sperimentale, I-16132 Genoa, Italy; Univ Genoa, Ctr Eccellenza Ric Biomed, I-16132 Genoa, Italy; Univ Genoa, Dipartimento Oncol Biol & Genet, I-16132 Genoa, Italy; Ist Nazl Ric Canc, I-16132 Genoa, Italy. IMMUNOLOGY LETTERS (5 AUG 2003) Vol. 88, No. 2, pp. 89-93. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0165-2478. Pub. country: Italy. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The capability of killing various tumors or cells infected by certain viruses is a property shared by natural killer (NK) cells and by a subset of cytolytic T lymphocytes (CTLs) termed NK-CTL. Recent analysis of the molecular basis in these phenomena, however, revealed rather different molecular mechanisms. Thus, while NK cell cytotoxicity is regulated by a complex balance between activating signals (delivered by non HLA-class I-specific triggering receptors) and inhibitory signals (delivered by HLA-class I-specific receptors) the effector function of NK-CTL reflects the TCR-mediated recognition of the poorly polymorphic HLA-E.

L42 ANSWER 6 OF 16 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

2003:617003 The Genuine Article (R) Number: 699AX. Human natural killer cell function and their interactions with dendritic cells. Moretta L (Reprint); Ferlazzo G; Mingari M C; Melioli G; **Moretta A.** Ist Giannina Gaslini, Lgo Gerolamo Gaslini 5, I-16148 Genoa, Italy (Reprint); Ist Giannina Gaslini, I-16148 Genoa, Italy; Univ Genoa, Dipartimento Med Sperimentale, I-16132 Genoa, Italy; Univ Genoa, Ctr Eccellenza Ric Biomed, I-13162 Genoa, Italy; Ist Nazl Ric Canc, I-16132 Genoa, Italy; Univ Genoa, Dipartimento Oncol Biol & Genet, I-16132 Genoa, Italy. VACCINE (1 JUN 2003) Vol. 21, Supp. [2], pp. S38-S42. Publisher: ELSEVIER SCI LTD. THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, OXON, ENGLAND. ISSN: 0264-410X. Pub. country: Italy. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Natural killer (NK) cells have long been considered as "primitive" and "non-specific" effector cells. However, the past 10 years have witnessed dramatic progress in our understanding of how NK cells function and their role in innate defenses. Thanks to specialized inhibitory receptors specific for MHC-class I molecules, they can sense the decrease or loss of these molecules, a typical condition of potentially dangerous cells such as tumor or virally-infected cells. NK cell triggering and lysis of these cells is mediated by several activating receptors and co-receptors that have recently been identified and cloned. While normal cells are usually resistant to the NK-mediated attack, a remarkable exception is represented by dendritic cells (DC). In their immature form (iDC), they are susceptible to NK-mediated lysis because of the expression of low levels of surface MHC-class I molecules. Since the process of DC maturation (mDC) is characterized by the surface expression of high levels of MHC-class I molecules, mDC become resistant to NK cells. Exposure to live bacteria induces rapid DC maturation and, thus, resistance to NK cells. The cross-talk between DC and NK cells is more complex and involves also a DC-dependent NK cell activation and proliferation. Thus, two important players of the innate immunity may be involved in a coordinated regulation of critical events occurring at the interface between innate and adaptive immunity.

L42 ANSWER 7 OF 16 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

2003:103151 The Genuine Article (R) Number: 635FR. **Human NK cells** and their receptors. Moretta L (Reprint); **Biassoni R; Bottino C;** Cantoni C; Pende D; Mingari M C; **Moretta A.** Univ Genoa, Dipartimento Med Sperimentale, Genoa, Italy (Reprint); Ist Giannina Gaslini, I-16148 Genoa, Italy; Univ Genoa, Ctr Eccellenza Ric Biomed, Genoa, Italy; Ist Nazl Ric Canc, I-16132 Genoa, Italy; Univ Genoa,

Dipartimento Oncol Biol & Genet, Genoa, Italy. MICROBES AND INFECTION (DEC 2002) Vol. 4, No. 15, pp. 1539-1544. Publisher: EDITIONS SCIENTIFIQUES MEDICALES ELSEVIER. 23 RUE LINOIS, 75724 PARIS CEDEX 15, FRANCE. ISSN: 1286-4579. Pub. country: Italy. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The past decade has witnessed important progress in our understanding of how natural killer (NK) cells function. This is primarily consequent to the identification and functional characterization of MHC-specific inhibitory receptors that allow NK cells to discriminate between normal cells and potentially harmful cells that have lost or express insufficient amounts of MHC class I molecules. More recently, a number of activating receptors or coreceptors have been identified that are involved in the process of natural cytotoxicity but may also play a role in the direct recognition of pathogen-associated structures. Surprisingly, none of the triggering receptors identified in NK cells appears to be involved in the "NK-like activity" of a subset of CD8(+) cytolytic T lymphocytes. In this case, lysis of NK-susceptible tumor target cells is the result of the TCR alpha/beta-mediated recognition of HLA-E. The potent cytolytic activity of NK cells as well as their unique mode of functioning may be exploited in therapy. An important breakthrough is the recent report that "alloreactive" NK cells, generated in haploidentical bone marrow transplantation in patients with acute myeloid leukemias, may efficiently prevent leukemic relapses as well as graft rejection and graft-vs.-host disease. This may lead to a true revolution in bone marrow transplantation, based on the exploitation of appropriate HLA-Cl I mismatches that can put NK cells in action.

L42 ANSWER 8 OF 16 MEDLINE on STN DUPLICATE 3
2001164248. PubMed ID: 11265639. Identification of NKp80, a novel triggering molecule expressed by **human NK cells**. Vitale M; Falco M; Castriconi R; Parolini S; Zambello R; Semenzato G; **Biassoni R; Bottino C**; Moretta L; **Moretta A**. (Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy.) European journal of immunology, (2001 Jan) 31 (1) 233-42. Journal code: 1273201. ISSN: 0014-2980. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB The ability of NK cells to kill a wide range of tumor or virally infected target cells as well as normal allogeneic T cell blasts appears to depend upon the concerted action of multiple triggering **NK receptors**. In this study, using two specific monoclonal antibodies [(mAb) MA152 and LAP171], we identified a triggering **NK receptor** expressed at the cell surface as a dimer of approximately 80 kDa (NKp80). NKp80 is expressed by virtually all fresh or activated NK cells and by a minor subset of T cells characterized by the CD56 surface antigen. NKp80 surface expression was also detected in all CD3- and in 6 / 10 CD3+ large granular lymphocyte expansions derived from patients with lymphoproliferative disease of granular lymphocytes. In polyclonal NK cells, mAb-mediated cross-linking of NKp80 resulted in induction of cytolytic activity and Ca2+ mobilization. A marked heterogeneity existed in the magnitude of the cytolytic responses of different NK cell clones to anti-NKp80 mAb. This heterogeneity correlated with the surface density of NKp46 molecules expressed by different NK clones. The mAb-mediated masking of NKp80 led to a partial inhibition of the NK-mediated lysis of appropriate allogeneic phytohemagglutinin-induced T cell blasts, while it had no effect on the lysis of different tumor target cells, including T cell leukemia cells. These data suggest that NKp80 recognizes a ligand on normal T cells that may be down-regulated during tumor transformation. Molecular cloning of the cDNA coding for NKp80 revealed a type II transmembrane molecule of 231 amino acids identical to the putative protein encoded by a recently identified cDNA termed KLRF1.

L42 ANSWER 9 OF 16 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2001:646708 The Genuine Article (R) Number: 460ZW. Human natural killer cell receptors and co-receptors. **Biassoni R (Reprint)**; Cantoni C; Pende D; Sivori S; Parolini S; Vitale M; **Bottino C**; **Moretta A**. CBA, IST, Immunol Lab, Lgo R Benzi 10, I-16132 Genoa, Italy (Reprint); Ist Nazl Ric Canc, I-16132 Genoa, Italy; Univ Genoa, Dipartimento Med Sperimentale, Genoa, Italy; Ist Giannina Gaslini, I-16148 Genoa, Italy; Univ Brescia, Dipartimento Sci Biomed & Biotechnol, I-25121 Brescia, Italy. IMMUNOLOGICAL REVIEWS (JUN 2001) Vol. 181, pp. 203-214. Publisher: MUNKSGAARD INT PUBL LTD. 35 NORRE SOGADE, PO BOX 2148, DK-1016 COPENHAGEN, DENMARK. ISSN: 0105-2896. Pub. country: Italy. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In the absence of sufficient signaling by their HLA class I-specific inhibitory receptors, human natural killer (NK) cells become activated and display potent cytotoxicity against cells that are either HLA class I negative or deficient. This indicates that the **NK receptors** responsible for the induction of cytotoxicity recognize ligands on target cells different from HLA class I molecules. On this basis, the process of NK-cell triggering can be considered as a mainly non-NMC-restricted mechanism. The recent identification of a group of NK-specific triggering surface molecules has allowed a first series of pioneering studies on the functional/molecular characteristics of such receptors. The first three members of a receptor family that has been termed natural cytotoxicity receptors (NCR) are represented by NKp46, NKp44 and NKp30. These receptors are strictly confined to NK cells, and their engagement induces a strong activation of NK-mediated cytolysis. A direct correlation exists between the surface density of NCR and the ability of NK cells to kill various target cells. Importantly, mAb-mediated blocking of these receptors has been shown to suppress cytotoxicity against most NK-susceptible target cells. However, the process of NK-cell triggering during target cell lysis may also depend on the concerted action of NCR and other triggering receptors, such as NKG2D, or surface molecules, including 2B4 and NKp80, that appear to function as co-receptors rather than as true receptors. Notably, a dysfunction of 2B4 has been associated with a severe form of immunodeficiency termed X-linked lymphoproliferative disease. Future studies will clarify whether also the altered expression and/or function of other NK-triggering molecules may represent a. possible cause of immunological disorders.

L42 ANSWER 10 OF 16 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2001:350708 The Genuine Article (R) Number: 424LW. Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. **Moretta A (Reprint)**; **Bottino C**; Vitale M; Pende D; Cantoni C; Mingari M C; **Biassoni R**; Moretta L. Univ Genoa, Dipartimento Med Sperimentale, Genoa, Italy (Reprint); Ist Nazl Ric Canc, I-16132 Genoa, Italy; Univ Genoa, Dipartimento Oncol Biol & Genet, Genoa, Italy; Inst G Geslini, Genoa, Italy. ANNUAL REVIEW OF IMMUNOLOGY (MAY 2001) Vol. 19, pp. 197-223. Publisher: ANNUAL REVIEWS. 4139 EL CAMINO WAY, PO BOX 10139, PALO ALTO, CA 94303-0139 USA. ISSN: 0732-0582. Pub. country: Italy. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Natural killer cells can discriminate between normal cells and cells that do not express adequate amounts of major histocompatibility complex (MHC) class I molecules. The discovery, both in mouse and in human, of MHC specific inhibitory receptors clarified the molecular basis of this important NK cell function. However, the triggering receptors responsible for positive NK cell stimulation remained elusive until recently. Some of these receptors have now been identified in humans, thus shedding some light on the molecular mechanisms involved in NK cell activation during the process of natural cytotoxicity. Three novel, NK-specific, triggering surface molecules (NKp46, NKp30, and NKp44) have been identified. They

represent the first members of a novel emerging group of receptors collectively termed natural cytotoxicity receptors (NCR). Monoclonal antibodies (mAbs) to NCR block to differing extents the NK-mediated lysis of various tumors. Moreover, lysis of certain tumors can be virtually abrogated by the simultaneous masking of the three NCRs. There is a coordinated surface expression of the three NCRs, their surface density varying in different individuals and also in the NK cells isolated from a given individual. A direct correlation exists between the surface density of NCR and the ability of NK cells to kill various tumors. NKp46 is the only NCR involved in human NK-mediated killing of murine target cells. Accordingly, a homologue of NKp46 has been detected in mouse. Molecular cloning of NCR revealed novel members of the Ig superfamily displaying a low degree of similarity to each other and to known human molecules. NCRs are coupled to different signal transducing adaptor proteins, including CD3 zeta, Fc epsilon RI gamma, and KARAP/DAP12. Another triggering **NK receptor** is NKG2D. It appears to play either a complementary or a synergistic role with NCRs. Thus, the triggering of NK cells in the process of tumor cell lysis may often depend on the concerted action of NCR and NKG2D. In some instances, however, it may uniquely depend upon the activity of NCR or NKG2D only. Strict NKG2D-dependency can be appreciated using clones that, in spite of their NCRdull phenotype, efficiently lyse certain epithelial tumors or leukemic cell lines. Other triggering surface molecules including 2B4 and the novel NKp80 appear to function as coreceptors rather than as true receptors. Indeed, they can induce natural cytotoxicity only when co-engaged with a triggering receptor. While an altered expression or function of NCR or NKG2D is being explored as a possible cause of immunological disorders, 2B4 dysfunction has already been associated with a severe form of immunodeficiency. indeed, in patients with the X-linked lymphoproliferative disease, the inability to control Epstein-Barr virus infections may be consequent to a major dysfunction of 2B4 that exerts inhibitory instead of activating functions.

L42 ANSWER 11 OF 16 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2000:630869 The Genuine Article (R) Number: 343NQ. X-linked lymphoproliferative disease: 2B4 molecules displaying inhibitory rather than activating function are responsible for the inability of natural killer cells to kill Epstein-Barr virus-infected cells. Parolini S; **Bottino C**; Falco M; Augugliaro R; Giliani S; Franceschini R; Ochs H D; Wolf H; Bonnefoy J Y; **Biassoni R**; Moretta L; Notarangelo L D; **Moretta A (Reprint)**. UNIV GENOA, DIPARTIMENTO MED SPERIMENTALE, SEZ ISTOL, VIA GB MARSANO 10, I-16132 GENOA, ITALY (Reprint); UNIV GENOA, DIPARTIMENTO MED SPERIMENTALE, SEZ ISTOL, I-16132 GENOA, ITALY; UNIV BRESCIA, DIPARTIMENTO SCI BIOMED & BIOTECNOL, I-25123 BRESCIA, ITALY; IST NAZL RIC CANC, I-16132 GENOA, ITALY; UNIV BRESCIA, PEDIAT CLIN, IST MED MOL ANGELO NOCIVELLI, I-25123 BRESCIA, ITALY; UNIV WASHINGTON, DEPT PEDIAT, SEATTLE, WA 98195; UNIV VIENNA, DEPT IMMUNOL, A-1090 VIENNA, AUSTRIA; CTR IMMUNOL PIERRE FABRE, F-74164 ST JULIEN GENEVOI, FRANCE. JOURNAL OF EXPERIMENTAL MEDICINE (7 AUG 2000) Vol. 192, No. 3, pp. 337-346. Publisher: ROCKEFELLER UNIV PRESS. 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021. ISSN: 0022-1007. Pub. country: ITALY; USA; AUSTRIA; FRANCE. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB 2B4 is a surface molecule involved in activation of the natural killer (NK) cell-mediated cytotoxicity. It binds a protein termed Src homology 2 domain-containing protein (SH2D1A) or signaling lymphocyte activation molecule (SLAM)-associated protein (SAP), which in rum has been proposed to function as a regulator of the 2B4-associated signal transduction pathway. In this study, we analyzed patients with X-linked lymphoproliferative disease (XLP), a severe inherited immunodeficiency characterized by critical mutations in the SH2D1A gene and by the inability to control Epstein-Barr Virus (EBV) infections. We show that, in

these patients, 2B4 not only fails to transduce triggering signals, but also mediates a sharp inhibition of the NK-mediated cytolysis. Other receptors involved in NK cell triggering, including CD16, NKp46, NKp44, and NKp30, displayed a normal functional capability. However, their activating function was inhibited upon engagement of 2B4 molecules. CD48, the natural ligand of 2B4, is highly expressed on the surface of EBV+ B cell, lines. Remarkably, NK cells from XLP patients could not kill EBV+ B cell lines. This failure was found to be the consequence of inhibitory signals generated by the interaction between 2B4 and CD48, as the antibody-mediated disruption of the 2B4-CD48 interaction restored lysis of EBV+ target cells lacking human histocompatibility leukocyte antigen (HLA) class I molecules. In the case of autologous or allogeneic (HLA class I+) EBV+ lymphoblastoid cell lines, restoration of lysis was achieved only by the simultaneous disruption of 2B4-CD48 and **NK receptor** -HLA class I interactions. Molecular analysis revealed that 2B4 molecules isolated from either XLP or normal NK cells were identical. As expected, in XLP-NK cells, 2B4 did not associate with SH2D1A, whereas similar to 2B4 molecules isolated from normal NK cells, it did associate with Src homology 2 domain-containing phosphatase 1.

L42 ANSWER 12 OF 16 MEDLINE on STN DUPLICATE 4

1999157958. PubMed ID: 10050671. The leukocyte Ig-like receptor (LIR)-1 for the cytomegalovirus UL18 protein displays a broad specificity for different HLA class I alleles: analysis of LIR-1 + NK cell clones. Vitale M; Castriconi R; Parolini S; Pende D; Hsu M L; Moretta L; Cosman D; **Moretta A.** (Istituto Nazionale per la Ricerca sul Cancro e Centro Biotecnologie Avanzate, Genova, Italy.) International immunology, (1999 Jan) 11 (1) 29-35. Journal code: 8916182. ISSN: 0953-8178. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Leukocyte Ig-like receptor (LIR)-1 is a member of the Ig superfamily which has been shown to bind the human cytomegalovirus MHC class I homologue UL-18 protein. In this study, we have analyzed the expression and function of LIR-1 in **human NK cells.** We show that LIR-1 is expressed by a subset of NK cells variable in size among different donors. When compared to the known HLA class I-specific **NK receptors,** the expression of LIR-1 was found to be partially overlapped with that of CD94-NKG2A or with that of killer inhibitory receptors (KIR) belonging to the Ig superfamily. The use of the soluble form of UL-18 molecule revealed, in double fluorescence analysis, a selective binding to LIR-1 + cells while no correlation was observed between expression of either KIR or CD94-NKG2A molecules and ability to bind UL18. We further determined whether LIR-1 could also function as receptor for HLA class I molecules. To this end, we assessed the capability of LIR-1 + NK cell clones of lysing HLA class I- target cells transfected with different class I alleles, including HLA-A, -B, -C and -G alleles. Data revealed that LIR-1 functions as a broad HLA class I-specific inhibitory receptor recognizing different alleles coded for by different HLA loci.

L42 ANSWER 13 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN

1998:403153 Document No. 129:135076 NKp44, a novel triggering surface molecule specifically expressed by activated natural killer cells, is involved in non-major histocompatibility complex-restricted tumor cell lysis. Vitale, Massimo; **Bottino, Cristina;** Sivori, Simona; Sanseverino, Lorenza; Castriconi, Roberta; Marcenaro, Emanuela; Augugliaro, Raffaella; Moretta, Lorenzo; **Moretta, Alessandro** (Inst. Naz. Ricerca Cancro Cent. Biotecnol. Avanzate, Genoa, 16132, Italy). Journal of Experimental Medicine, 187(12), 2065-2072 (English) 1998. CODEN: JEMEAV. ISSN: 0022-1007. Publisher: Rockefeller University Press.

AB After culture in interleukin (IL)-2, natural killer (NK) cells acquire an increased capability of mediating non-major histocompatibility complex

(MHC)-restricted tumor cell lysis. This may reflect, at least in part, the de novo expression by NK cells of triggering receptors involved in cytolysis. In this study we identified a novel 44 kDa surface mol. (NKp44) that is absent in freshly isolated peripheral blood lymphocytes but is progressively expressed by all NK cells in vitro after culture in IL 2. Different from other markers of cell activation such as CD69 or VLA.2, NKp44 is absent in activated T lymphocytes or T cell clones. Since NKp44 was not detected in any of the other cell lineages analyzed, it appears as the first marker specific for activated **human NK cells**. Monoclonal antibody (mAb)-mediated crosslinking of NKp44 in cloned NK cells resulted in strong activation of target cell lysis in a redirected killing assay. This data indicated that NKp44 can mediate triggering of NK cell cytotoxicity. MAb-mediated masking of NKp44 resulted in partial inhibition of cytolytic activity against certain (FcγR neg.) NK-susceptible target cells. This inhibition was greatly increased by the simultaneous masking of p46, another recently identified NK-specific triggering surface mol. These data strongly suggest that NKp44 functions as a triggering receptor selectively expressed by activated NK cells that, together with p46, may be involved in the process of non-MHC-restricted lysis. Finally, we show that p46 and NKp44 are coupled to the intracytoplasmic transduction machinery via the association with CD3ζ or KARAP/DAP12, resp.; these associated mols. are tyrosine phosphorylated upon NK cell stimulation.

L42 ANSWER 14 OF 16 MEDLINE on STN DUPLICATE 5

1998401029. PubMed ID: 9730896. Molecular cloning of NKp46: a novel member of the immunoglobulin superfamily involved in triggering of natural cytotoxicity. Pessino A; Sivori S; **Bottino C**; Malaspina A; Morelli L; Moretta L; **Biassoni R**; **Moretta A**. (Dipartimento di Medicina Sperimentale, Universita degli Studi di Genova, Italy.) Journal of experimental medicine, (1998 Sep 7) 188 (5) 953-60. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB NKp46 has been shown to represent a novel, natural killer (NK) cell-specific surface molecule, involved in **human NK cell** activation. In this study, we further analyzed the role of NKp46 in natural cytotoxicity against different tumor target cells. We provide direct evidence that NKp46 represents a major activating receptor involved in the recognition and lysis of both human and murine tumor cells. Although NKp46 may cooperate with other activating receptors (including the recently identified NKp44 molecule) in the induction of NK-mediated lysis of human tumor cells, it may represent the only human **NK receptor** involved in recognition of murine target cells. Molecular cloning of the cDNA encoding the NKp46 molecule revealed a novel member of the immunoglobulin (Ig) superfamily, characterized by two C2-type Ig-like domains in the extracellular portion. The transmembrane region contains the positively charged amino acid Arg, which is possibly involved in stabilizing the association with CD3zeta chain. The cytoplasmic portion, spanning 30 amino acids, does not contain immunoreceptor tyrosine-based activating motifs. Analysis of a panel of human/hamster somatic cell hybrids revealed segregation of the NKp46 gene on human chromosome 19. Assessment of the NKp46 mRNA expression in different tissues and cell types unambiguously confirmed the strict NK cell specificity of the NKp46 molecule. Remarkably, in line with the ability of NKp46 to recognize ligand(s) on murine target cells, the cDNA encoding NKp46 was found to be homologous to a cDNA expressed in murine spleen. In conclusion, this study reports the first characterization of the molecular structure of a NK-specific receptor involved in the mechanism of NK cell activation during natural cytotoxicity.

L42 ANSWER 15 OF 16 MEDLINE on STN DUPLICATE 6

96100669. PubMed ID: 7579196. Receptors for HLA class I molecules in

human NK cells. Bottino C; Vitale M; Pende D; **Biassoni R; Moretta A.** (Centro di Biotecnologie Avanzate, Genova, Italy.) Seminars in immunology, (1995 Apr) 7 (2) 67-73. Ref: 23. Journal code: 9009458. ISSN: 1044-5323. Pub. country: United States. Language: English.

AB Recent studies have shown that NK cells recognize HLA-class I molecules. Moreover, the analysis of NK cell clones has provided evidence that they are capable of discriminating between different groups of HLA alleles. HLA class I recognition generates a negative signal which inhibits the NK cell cytotoxicity, thus resulting in target cell protection. HLA-class I recognition is mediated by clonally distributed receptors, some of which have been identified, characterized and cloned. The first two identified receptors were shown to be specific for HLA-C alleles, each recognizing a group of alleles sharing two amino acidic positions (77 and 80) in the peptide binding groove. The HLA-C specific receptors are represented by two 58 Kd (p58) molecules that are highly homologous, as shown by both biochemical analysis and by the comparison of the corresponding genes. Two additional receptors have been recently identified, which recognize two distinct groups of HLA-B alleles. These receptors are represented by the CD94 and by the NKB1 molecules, recognizing the Bw6 and Bw4 supertypic specificities. Recent analysis of the surface receptors involved in NK cell triggering has provided evidence that class I specific **NK receptors** can, in some instances, induce NK cell triggering, thus contributing to the activatory pathway of NK cells.

L42 ANSWER 16 OF 16 MEDLINE on STN DUPLICATE 7

93340636. PubMed ID: 8340759. P58 molecules as putative receptors for major histocompatibility complex (MHC) class I molecules in human natural killer (NK) cells. Anti-p58 antibodies reconstitute lysis of MHC class I-protected cells in NK clones displaying different specificities. **Moretta A;** Vitale M; **Bottino C;** Orengo A M; Morelli L; Augugliaro R; Barbaresi M; Ciccone E; Moretta L. (Istituto di Istologia ed Embriologia Generale, Universita di Genova, Italy.) Journal of experimental medicine, (1993 Aug 1) 178 (2) 597-604. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB Human CD3-16+56+ natural killer (NK) cells have been shown to display a clonally distributed ability to recognize major histocompatibility complex (MHC) class I alleles. Opposite to T lymphocytes, in NK cells, specific recognition of MHC class I molecules appears to induce inhibition of cytolytic activity and, thus, to protect target cells. Since a precise correlation has been established between the expression of the NK-specific GL183 and EB6 surface molecules (belonging to the novel p58 molecular family) and the specificity of NK clones, we analyzed whether p58 molecules could function as receptors for MHC in **human NK cells**. NK clones displaying the previously defined "specificity 2" and characterized by the GL183+EB6+ phenotype, specifically recognize the Cw3 allele and thus fail to lyse the Fc gamma R+ P815 target cells transfected with Cw3. On the other hand, NK clones displaying "specificity 1" and expressing the GL183-EB6+ phenotype failed to lyse Cw4+ target cells. Addition of the F(ab')2 fragments of either GL183 or EB6 mAb as well as the XA141 mAb of IgM isotype (specific for the EB6 molecules) completely restored the lysis of Cw3-transfected P815 cells by the Cw3-specific NK clones EX2 and EX4. Similarly, both the entire EB6 mAb, its F(ab')2 fragment and the XA141 mAb reconstituted the lysis of C1R, a Fc gamma R- target cell expressing Cw4 as the only serologically detected class I antigen. Thus, it appears that masking of different members of p58 molecules prevents recognition of "protective" MHC class I alleles and thus the delivering of inhibitory signals. Further support to the concept that p58 molecules represent a **NK receptor** delivering a negative signal was provided by experiments in which the entire anti-p58 mAbs (of IgG isotype) could inhibit the lysis of unprotected Fc gamma R+ P815 target cells, thus mimicking the inhibitory